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L50: Entry 46 of 69 File: USPT Aug 24, 1999

DOCUMENT-IDENTIFIER: US 5942230 A
TITLE: Composition of immunotoxins and retinoids and use thereof

Detailed Description Text (78):
The effects of retinoic acid on ricin, diphtheria toxin, and Pseudomonas exotoxin are consistent with this model that retinoic acid alters the endocytotic routing in cells. Native ricin, modeccin and abrin toxicity are blocked a small amount by 10 .mu.M retinoic acid in HeLa cells and Vero cells (Sandvig, K., et al. (1981) Biochem. J. 194:821-827). Retinoic acid also protects U251 cells from ricin toxicity (data not shown). These results and the potentiation of ricin A chain immunotoxins by retinoic acid are similar to the effects seen with lysosomotropic amides and ionophores. Native ricin contains a B chain that is thought to use a galactose binding function, intracellularly, to route the toxin through the Golgi apparatus to reach the cytosol (Johnson, V. G., et al. (1991) "Intracellular Routing and membrane translocation of diphtheria toxin and ricin" In Intracellular Trafficking of Proteins: 183-225 Steer and Hanover (eds) (Karger/Basel). The galactose binding activity may allow ricin, in the trans-Golgi, to bind to KDEL receptor-like glycoproteins that cycle to and from the cis-Golgi and the endoplasmic reticulum (ER) (Zhang, X. K., et al. (1992) Nature 355:441-446). Immunotoxins that lack a B chain are much less potent, apparently due to a deficiency in intracellular routing. Ricin A chain immunotoxins may recycle through the trans-Golgi back to the cell surface repeatedly in the absence of a B chain and disruption of the Golgi with monensin or retinoic acid may allow trans-Golgi to cis-Golgi movement, sensitizing the cell to the toxin. Retinoic acid, by disrupting the Golgi, may slightly disrupt the efficient B chain mechanism of Golgi transport resulting in a small inhibition of native ricin toxicity while potentiating the inefficient ricin A chain immunotoxin routing.

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L6: Entry 14 of 23 File: USPT Mar 25, 1997

DOCUMENT-IDENTIFIER: US 5614488 A
TITLE: Epidermal growth factor receptor targeted molecules for treatment of inflammatory arthritis

CLAIMS:

1. A method for treating a patient having an inflammatory arthritis characterized by by higher than normal expression of the epidermal growth factor receptor on synovial synovial fibroblasts, said method comprising administering to said patient a hybrid molecule comprising a first portion and a second portion joined together covalently, said first portion comprising fragment A of diphtheria toxin and enough of fragment B of diphtheria toxin to facilitate entry of said hybrid molecule into the cytosol of a cell to which said hybrid molecule binds, said second portion comprising all or an epidermal growth factor receptor-binding portion of epidermal growth factor.

4. A method for treating a patient having an inflammatory arthritis characterized by higher than normal expression of the epidermal growth factor receptor on synovial synovial fibroblasts, said method comprising administering to said patient a hybrid molecule comprising a first portion and a second portion joined together covalently, said first portion comprising fragment A of diphtheria toxin and enough of fragment B of diphtheria toxin to facilitate entry of said hybrid molecule into the cytosol of a cell to which said hybrid molecule binds, said second portion comprising all or an epidermal growth factor receptor-binding portion of tissue growth factor alpha.

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L43: Entry 10 of 15 File: USPT Oct 6, 1998

US-PAT-NO: 5817512
DOCUMENT-IDENTIFIER: US 5817512 A
**** See image for Certificate of Correction ****

TITLE: Encapsidated recombinant viral nucleic acid and methods of making and using same

DATE-ISSUED: October 6, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Porter; Donna C.	Birmingham	AL		
Ansardi; David C.	Warrior	AL		

US-CL-CURRENT: 435/320.1; 424/199.1, 424/217.1, 435/456, 435/465, 435/69.3

CLAIMS:

What is claimed is:

1. An encapsidated recombinant poliovirus nucleic acid which does not express proteins sufficient for encapsidation, wherein the encapsidated poliovirus nucleic acid

is substantially free of nucleic acid which competes with the encapsidated recombinant poliovirus nucleic acid for encapsidation by proteins sufficient for encapsidation of the recombinant poliovirus nucleic acid; and

includes a nucleotide sequence which encodes amino acids which allow for processing of a foreign protein or fragment thereof encoded by a foreign nucleotide sequence inserted in the poliovirus nucleic acid.

2. A method for encapsidating a recombinant poliovirus nucleic acid, comprising comprising the steps of:

providing

a recombinant poliovirus nucleic acid which does not express proteins sufficient for encapsidation, the recombinant poliovirus nucleic acid further comprising a nucleotide sequence which encodes amino acids which allow for processing of a foreign protein or fragment thereof encoded by a foreign nucleotide sequence inserted in the poliovirus nucleic acid; and

an expression vector the nucleic acid of which does not compete with the recombinant poliovirus nucleic acid for encapsidation by proteins sufficient for encapsidation of the recombinant poliovirus nucleic acid and which encodes

at least a portion of a protein necessary for encapsidating the recombinant poliovirus nucleic acid and directs expression of at least a portion of a protein necessary for encapsidating the recombinant poliovirus nucleic acid; and

contacting a host cell with the recombinant poliovirus nucleic acid and the expression vector under conditions appropriate for introduction of the recombinant poliovirus nucleic acid and the expression vector into the host cell; and

obtaining a yield of encapsidated viruses which substantially comprises encapsidated recombinant poliovirus nucleic acid.

3. The encapsidated recombinant poliovirus nucleic acid of claim 1, which lacks lacks the nucleotide sequence encoding at least a portion of the capsid precursor protein P1.

4. The encapsidated recombinant poliovirus nucleic acid of claim 1, which lacks lacks the nucleotide sequence encoding at least a portion of the capsid proteins VP1 and VP2, VP1 and VP3, VP1 and VP4, VP2 and VP3, VP2 and VP4, or VP3 and VP4.

5. The encapsidated recombinant poliovirus nucleic acid of claim 1, which lacks lacks the nucleotide sequence encoding the entire capsid precursor protein P1.

6. The encapsidated recombinant poliovirus nucleic acid of claim 1, wherein a nucleotide sequence of the encapsidated recombinant poliovirus nucleic acid which encodes at least a portion of a protein necessary for encapsidating the recombinant poliovirus nucleic acid is replaced by a foreign nucleotide sequence encoding, in an expressible form, a foreign protein or fragment thereof.

7. The encapsidated recombinant poliovirus nucleic acid of claim 1, wherein the the amino acids which allow for processing of the foreign protein or fragment comprise a cleavage site for an enzyme.

8. The encapsidated recombinant poliovirus nucleic acid of claim 7, wherein the the enzyme is poliovirus 2A protease.

9. The encapsidated recombinant poliovirus nucleic acid of claim 1, wherein the the amino acids which allow for processing of the foreign protein or fragment comprise spacing for processing of the foreign protein or fragment thereof.

10. The encapsidated recombinant poliovirus nucleic acid of claim 6, wherein the foreign nucleotide sequence encodes a protein or fragment thereof selected from the group consisting of a viral antigen or a fragment thereof, a bacterial bacterial antigen or a fragment thereof, a tumor antigen or a fragment thereof, thereof, an immunological response modifier or a fragment thereof, and a protein with enzymatic activity or a fragment thereof.

11. The encapsidated recombinant poliovirus nucleic acid of claim 10, wherein the viral antigen or fragment thereof is selected from the group consisting of a human immunodeficiency viral antigen or a fragment thereof, a hepatitis viral viral antigen or a fragment thereof, an influenza viral antigen or a fragment thereof, a respiratory syncytial viral antigen or a fragment thereof, and a rotaviral antigen or a fragment thereof.

12. The encapsidated recombinant poliovirus nucleic acid of claim 11, wherein the human immunodeficiency viral antigen is selected from the group consisting of the gag protein or a fragment thereof, the pol protein or a fragment thereof, and the env protein or a fragment thereof.

13. The encapsidated recombinant poliovirus nucleic acid of claim 10, wherein the bacterial antigen or fragment thereof is selected from the group consisting of tetanus toxin or a fragment thereof, diphtheria toxin or a fragment thereof, cholera toxin or a fragment thereof, mycobacterium tuberculosis protein antigen B or a fragment thereof, and a protein from Helicobactor pylori or a fragment thereof.

14. The encapsidated recombinant poliovirus nucleic acid of claim 10, wherein the tumor antigen or fragment thereof is selected from the group consisting of carcinoembryonic antigen or fragment thereof, melanoma ganglioside antigen GM2 or a fragment thereof, melanoma ganglioside antigen GD2 or a fragment thereof, melanoma ganglioside antigen GD3 or a fragment thereof, antigen Jen CRG from colorectal and lung cancer cells or a fragment thereof, a synthetic peptide of immunoglobulin epitope from B cell malignancies or a fragment thereof, and an antigen which is a product of the oncogene erb, neu, or sis.

15. The encapsidated recombinant poliovirus nucleic acid of claim 10, wherein the immunological response modifier or fragment thereof is a cytokine or fragment thereof or a linear B or T cell epitope or a fragment thereof.

16. The encapsidated recombinant poliovirus nucleic acid of claim 6, wherein the foreign nucleotide sequence encodes a ribozyme.

17. The encapsidated recombinant poliovirus nucleic acid of claim 6, wherein the foreign nucleotide sequence encodes an antisense nucleic acid.

18. An immunogenic composition comprising the recombinant poliovirus nucleic acid of claim 1 and a physiologically acceptable carrier.

19. An immunogenic composition comprising the recombinant poliovirus nucleic acid of claim 6 and a physiologically acceptable carrier.

20. The method of claim 2 wherein the expression vector is introduced into the host cell prior to the introduction of the recombinant poliovirus nucleic acid. acid.

21. The method of claim 2 wherein a nucleotide sequence of the encapsidated recombinant poliovirus nucleic acid which encodes at least a portion of a protein necessary for encapsidating the recombinant poliovirus nucleic acid is replaced by a foreign nucleotide sequence encoding, in an expressible form, a foreign protein or fragment thereof.

22. The method of claim 2 wherein the expression vector comprises a virus.

23. The method of claim 22 wherein the virus is a recombinant vaccinia virus.

24. The method of claim 2 wherein the expression vector comprises a plasmid.

25. The method of claim 2, wherein the amino acids which allow for processing of the foreign protein or fragment comprise a cleavage site for an enzyme.

26. The method of claim 25, wherein the enzyme is poliovirus 2A protease.

27. The method of claim 2, wherein the amino acids which allow for processing of the foreign protein or fragment provide spacing for processing of the foreign protein or fragment thereof.

28. The method of claim 21, wherein the foreign nucleotide sequence encodes a protein or fragment thereof selected from the group consisting of a viral antigen or a fragment thereof, a bacterial antigen or a fragment thereof, a tumor antigen or a fragment thereof, an immunological response modifier or a fragment thereof, and a protein with enzymatic activity or a fragment thereof.

29. The method of claim 28, wherein the viral antigen or fragment thereof is selected from the group consisting of a human immunodeficiency viral antigen or or a fragment thereof, a hepatitis viral antigen or a fragment thereof, an influenza viral antigen or a fragment thereof, a respiratory syncytial viral antigen or a fragment thereof, and a rotaviral antigen or a fragment thereof.

30. The method of claim 29, wherein the human immunodeficiency viral antigen or or fragment thereof is selected from the group consisting of the gag protein or or a fragment thereof, the pol protein or a fragment thereof, and the env protein or a fragment thereof.

31. The method of claim 28, wherein the bacterial antigen or fragment thereof is selected from the group consisting of tetanus toxin or a fragment thereof, diphtheria toxin or a fragment thereof, cholera toxin or a fragment thereof, mycobacterium tuberculosis protein antigen B or a fragment thereof, and a protein from Helicobactor pylori or a fragment thereof.

32. The method of claim 28, wherein the tumor antigen or fragment thereof is selected from the group consisting of carcinoembryonic antigen or a fragment thereof, melanoma ganglioside antigen GM2 or a fragment thereof, melanoma ganglioside antigen GD2 or a fragment thereof, melanoma ganglioside antigen GD3 GD3 or a fragment thereof, antigen Jen CRG from colorectal and lung cancer cells or a fragment thereof, a synthetic peptide of immunoglobulin epitope from from B cell malignancies or a fragment thereof, and an antigen which is a product of the oncogene erb, neu, or sis.

33. The method of claim 28, wherein the immunological response modifier or fragment thereof is a cytokine or fragment thereof or a linear B or T cell epitope or fragment thereof.

34. The method of claim 21, wherein the foreign nucleotide sequence encodes a ribozyme.

35. The method of claim 21, wherein the foreign nucleotide sequence encodes an antisense nucleic acid.

36. The method of claim 2 wherein the host cell is a mammalian host cell.

37. An encapsidated recombinant poliovirus nucleic acid produced by the method of claim 2.

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The invention also includes vectors (e.g., plasmids, phages and viruses) including DNA sequences encoding the diphtheria toxoid mutants described herein. Expression of a diphtheria toxoid polypeptide of the invention can be under the control of a heterologous promoter, and/or the expressed amino acids can be linked to a signal sequence. A "heterologous promoter" is a promoter region that is not identical to the promoter region found in a naturally occurring diphtheria toxin gene. The promoter region is a segment of DNA 5' to the transcription start site of a gene, to which RNA polymerase binds before initiating transcription of the gene. Nucleic acids encoding a diphtheria toxoid of the invention can be prepared as an essentially pure preparation, which is a preparation that is substantially free of other nucleic acid molecules with which a nucleic acid encoding diphtheria toxin is naturally associated in Corynebacterium. A nucleic acid encoding a diphtheria toxoid of the invention can be contained in a cell, or a homogeneous population of cells, preferably a B. subtilis, Bacillus Calmette-Guerin (BCG), Salmonella sp., Vibrio cholerae, Corynebacterium diphtheriae, Listeriae, Yersiniae, Streptococci, or E. coli cell. The cell is capable of expressing the diphtheria toxoid polypeptide of the invention.

12 Diphtheria toxoids that are "immunologically cross-reactive" possess at least one antigenic determinant in common with naturally occurring diphtheria toxin, so that they are each bound by at least one antibody with specificity for naturally occurring diphtheria toxin. A diphtheria toxoid of the invention is immunologically cross-reactive with naturally occurring diphtheria toxin and possesses at least one of the mutations described herein.

13 The invention includes various vaccines that can be used to immunize a mammal (e.g., a human) against progression of the disease diphtheria, and against infection by the bacterium Corynebacterium diphtheriae. A vaccine of the invention can include any of the various DNAs encoding a diphtheria toxoid of the invention. Alternatively, a cell or virus expressing a nucleic acid of the invention, e.g., a live vaccine cell, can be used as a vaccine. Examples of suitable cells include B. subtilis, BCG, Salmonella sp., Vibrio cholerae, Listeriae, Yersiniae, Streptococci, Corynebacterium diphtheriae, and E. coli. A "live vaccine cell" can be a naturally avirulent live microorganism, or a live microorganism with low or attenuated virulence, that expresses an immunogen. A killed-cell vaccine can also be used.

14 One method for manufacturing a vaccine of the invention includes culturing a cell containing DNA encoding a diphtheria toxoid of the invention under conditions permitting proliferation of the cell and expression of the DNA, the cell being one that is suitable for introduction into an animal as a live-cell vaccine. The vaccine can be used in a method of immunizing a mammal against diphtheria by introducing an immunizing amount of a vaccine of the invention into the mammal.

15 In an alternative method of vaccination, an acellular vaccine that includes a nucleic acid encoding a diphtheria toxoid of the invention is introduced into the mammal. For example, a DNA vaccine can be administered by biolistic transfer, a method of delivery involving coating a microprojectile with DNA encoding an immunogen of interest, and injecting the coated microprojectile directly into cells of the recipient (Tang et al., Nature 356:152-154, 1992; hereby incorporated by reference). The diphtheria toxoid of the invention is then expressed from the DNA to stimulate an immune response in the recipient.

16 The polypeptides can be made by any of a variety of conventional methods, such as by culturing any of the various cells containing a DNA encoding a diphtheria toxoid of the invention under conditions permitting expression of the DNA. Included in the invention is an isolated mutant diphtheria toxin

polypeptide, an "isolated" polypeptide being one that is substantially free of cellular material, viral material, culture medium (when produced by recombinant recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Generally, the polypeptide is a substantially pure preparation, meaning that at least 50% (by weight) (e.g., at least 75%, 90%, or or 99%) of the protein present in the preparation is the diphtheria toxoid polypeptide of the

L39: Entry 21 of 33 File: USPT Nov 9, 1999

US-PAT-NO: 5980898
DOCUMENT-IDENTIFIER: US 5980898 A

TITLE: Adjuvant for transcutaneous immunization

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Alving; Carl R.	Bethesda	MD		

US-CL-CURRENT: 424/184.1; 424/240.1, 424/241.1, 424/275.1, 424/449, 424/450, 424/85.1, 530/363, 530/403

CLAIMS:

What we claim is:

1. A patch for transcutaneous immunization comprising:

(a) a dressing, said dressing being configured to cause hydration of intact skin;

(b) an immunizing antigen, and

(c) an adjuvant; whereby application of the patch to said intact skin induces an immune response specific for said immunizing antigen.

2. The patch of claim 1, wherein the dressing is an occlusive dressing.

3. The patch of claim 1, wherein exposure of a Langerhans cell to the adjuvant activates the Langerhans cell.

4. The patch of claim 1, wherein exposure of a Langerhans cell to the adjuvant causes migration of the Langerhans cell to a lymph node.

5. The patch of claim 1, wherein exposure of a Langerhans cell to the adjuvant signals the Langerhans cell to mature into a dendritic cell.

6. The patch of claim 1, wherein the adjuvant is an ADP-ribosylating exotoxin.

7. The patch of claim 6, wherein the adjuvant is cholera toxin (CT) or cholera toxin B subunit (CTB).

8. The patch of claim 6, wherein the adjuvant is E. coli heat-labile

enterotoxin (LT) or pertussis toxin.

9. The patch of claim 1, wherein the patch covers more than one draining lymph node field.

10. The patch of claim 1, wherein the adjuvant has a molecular mass greater than 500 daltons.

11. The patch of claim 1, wherein the adjuvant is one of the members selected from the group consisting of bacterial DNA, cytokines, chemokines, tumor necrosis factor alpha, genetically altered toxins, chemically conjugated bacterial ADP ribosylating exotoxins (bAREs) and lipopolysaccharides.

12. The patch of claim 1, wherein the adjuvant has a molecular mass greater than 800 daltons.

13. The patch of claim 1, wherein the adjuvant has a molecular mass greater than 1000 daltons.

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L20: Entry 7 of 32 File: USPT May 27, 2003

US-PAT-NO: 6569463
DOCUMENT-IDENTIFIER: US 6569463 B2

TITLE: Solid carriers for improved delivery of hydrophobic active ingredients in pharmaceutical compositions

DATE-ISSUED: May 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Chen; Feng-Jing	Salt Lake City	UT		

US-CL-CURRENT: 424/497; 424/422, 424/427, 424/430, 424/433, 424/434, 424/435, 424/436, 424/441, 424/451, 424/457, 424/463, 424/464, 424/465, 424/466, 424/470, 424/474, 424/476, 424/482, 424/489, 424/490, 424/498, 514/773, 514/779, 514/784, 514/785, 514/786

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L20: Entry 7 of 32 File: USPT May 27, 2003

DOCUMENT-IDENTIFIER: US 6569463 B2
TITLE: Solid carriers for improved delivery of hydrophobic active ingredients in pharmaceutical compositions

Abstract Text (1):
The present invention provides solid pharmaceutical compositions for improved delivery of a wide variety of pharmaceutical active ingredients contained therein or separately administered. In one embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier including a substrate and an encapsulation coat on the substrate. The encapsulation coat can include different combinations of pharmaceutical active ingredients, hydrophilic surfactant, lipophilic surfactants and triglycerides. In another embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier being formed of different combinations of pharmaceutical active ingredients, hydrophilic surfactants, lipophilic surfactants and triglycerides. The compositions of the present invention can be used for improved delivery of hydrophilic or hydrophobic pharmaceutical active ingredients, such as drugs, nutrionals, cosmeceuticals and diagnostic agents.

Brief Summary Text (2):
The present invention relates to pharmaceutical delivery systems for pharmaceutical active ingredients, such as drugs, nutritionals, cosmeceuticals, and diagnostic agents. In particular, the present invention provides compositions and dosage forms including solid carriers for improved delivery of pharmaceutical active ingredients. ingredients.

Brief Summary Text (6):
Solid carriers for pharmaceutical active ingredients offer potential advantages over over micronized drugs, emulsions or solubilized formulations. Solid carriers, typically of size less than about 2 mm, can easily pass through the stomach, thus making the performance less prone to gastric emptying variability. Further, the problems of leakage and other disadvantages of liquid formulations are not present in solid carrier formulations. To date, however, such solid carrier formulations generally have been limited to a few specific drugs, due to difficulties in formulating appropriate drug/excipient compositions to effectively coat the active ingredient onto a carrier particle.

Brief Summary Text (21):
In one embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier including a substrate and an encapsulation coat on the substrate. The encapsulation coat includes at least one ionic or non-ionic hydrophilic surfactant. Optionally, the encapsulation coat can include a pharmaceutical active ingredient, a lipophilic component such as a lipophilic surfactant or a triglyceride, or both a pharmaceutical active ingredient and a lipophilic component.

Brief Summary Text (22):
In another embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier including a substrate and an encapsulation coat on the

substrate. The encapsulation coat includes a lipophilic component, such as a lipophilic surfactant or a triglyceride. Optionally, the encapsulation coat can include a pharmaceutical active ingredient, an-ionic or non-ionic hydrophilic surfactant, or both a pharmaceutical active ingredient and a hydrophilic surfactant. surfactant.

Brief Summary Text (23):
In another embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier including a substrate and an encapsulation coat on the substrate. The encapsulation coat includes a pharmaceutical active ingredient and an ionic or non-ionic hydrophilic surfactant; a pharmaceutical active ingredient and a lipophilic component such as a lipophilic surfactant or a triglyceride; or a pharmaceutical active ingredient and both a hydrophilic surfactant and a lipophilic component.

Brief Summary Text (24):
In another embodiment, the solid pharmaceutical composition includes a solid carrier, wherein the solid carrier is formed of at least two components selected from the group consisting of pharmaceutical active ingredients; ionic or non-ionic hydrophilic surfactants; and lipophilic components such as lipophilic surfactants and triglycerides.

Drawing Description Text (2):
In order to illustrate the manner in which the above-recited and other advantages and objects of the invention are obtained, a more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which are illustrated in the appended drawings. Understanding that these drawings depict only typical embodiments of the invention and are not therefore to be considered to be limiting of its scope, the invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Detailed Description Text (2):
The present invention provides solid pharmaceutical compositions for improved delivery of a wide variety of pharmaceutical active ingredients contained therein or separately administered. In one embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier including a substrate and an encapsulation coat on the substrate. The encapsulation coat can include different combinations of pharmaceutical active ingredients, hydrophilic surfactant, lipophilic surfactants and triglycerides. In another embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier being formed of different combinations of pharmaceutical active ingredients, hydrophilic surfactant, lipophilic surfactants and triglycerides. These and other embodiments, as well as preferred aspects thereof, are described in more detail below.

Detailed Description Text (5):
In the embodiments of the present invention which include active ingredients, the active ingredients suitable for use in the pharmaceutical compositions and methods of the present invention are not particularly limited, as the compositions are surprisingly capable of effectively delivering a wide variety of active ingredients. The active ingredient can be hydrophilic, lipophilic, amphiphilic or hydrophobic, and can be solubilized, dispersed, or partially solubilized and dispersed, in the encapsulation coat. Alternatively, the active ingredient can be provided separately from the solid pharmaceutical composition, such as for co-administration. Such active ingredients can be any compound or mixture of compounds having therapeutic or other value when administered to an animal, particularly to a mammal, such as drugs, nutrients, cosmeceuticals, diagnostic agents, nutritional agents, and the like. It should be appreciated that the categorization of an active ingredient as hydrophilic or hydrophobic may change, depending upon the particular salts, isomers, analogs and derivatives used.

Detailed Description Text (6):
In one embodiment, the active ingredient agent is hydrophobic. Hydrophobic active ingredients are compounds with little or no water solubility. Intrinsic water solubilities (i.e., water solubility of the unionized form) for hydrophobic active ingredients are less than about 1% by weight, and typically less than about 0.1% or 0.01% by weight. In a particular aspect of this embodiment, the active ingredient is is a hydrophobic drug. In other particular aspects, the active ingredient is a nutrient, a cosmeceutical, a diagnostic agent or a nutritional agent.

Detailed Description Text (12):
In another embodiment, the active ingredient is hydrophilic. Amphiphilic compounds are also included within the class of hydrophilic active ingredients. Apparent water solubilities for hydrophilic active ingredients are greater than about 0.1% by weight, and typically greater than about 1% by weight. In a particular aspect of this embodiment, the hydrophilic active ingredient is a hydrophilic drug. In other particular aspects, the hydrophilic active ingredient is a cosmeceutical, a diagnostic agent, or a nutritional agent.

Detailed Description Text (14):
Likewise, the hydrophilic active ingredient can be a cytokine, a peptidomimetic, a peptide, a protein, a toxoid, a serum, an antibody, a vaccine, a nucleoside, a nucleotide, a portion of genetic material, a nucleic acid, or a mixture thereof.

Detailed Description Text (15):
Specific, non-limiting examples of suitable hydrophilic active ingredients include: acarbose; acyclovir; acetyl cysteine; acetylcholine chloride; alatrofloxacin; alendronate; aglucerase; amantadine hydrochloride; ambenomium; amifostine; amiloride hydrochloride; aminocaproic acid; amphotericin B; antihemophilic factor (human), antihemophilic factor (porcine); antihemophilic factor (recombinant), aprotinin; asparaginase; atenolol; atracurium besylate; atropine; azithromycin; aztreonam; BCG vaccine; bacitracin; becalermin; belladona; bepridil hydrochloride; bleomnycin sulfate; calcitonin human; calcitonin salmon; carboplatin; capecitabine; capreomycin sulfate; cefamandole nafate; cefazolin sodium; cefepime hydrochloride; cefixime; cefonicid sodium; cefoperazone; cefotetan disodium; cefotaxime; cefoxitin sodium; ceftizoxime; ceftriaxone; cefuroxime axetil; cephalexin; cephapirin sodium; cholera vaccine; chorionic gonadotropin; cidofovir; cisplatin; cladribine; clidinium clidinium bromide; clindamycin and clindamycin derivatives; ciprofloxacin; clodronate; colistimethate sodium; colistin sulfate; corticotropin; cosyntropin; cromolyn sodium; cytarabine; dalteparin sodium; danaparoid; desferrioxamine; denileukin diflitox; desmopressin; diatrizoate meglumine and diatrizoate sodium; dicyclomine; didanosine; dirithromycin; dopamine hydrochloride; dornase alpha; doxacurium chloride; doxorubicin; etidronate disodium; enalaprilat; enkephalin; enoxaparin; enoxaprin sodium; ephedrine; epinephrine; epoetin alpha; erythromycin; esmolol hydrochloride; factor IX; famciclovir; fludarabine; fluoxetine; foscarnet sodium; ganciclovir; granulocyte colony stimulating factor, granulocyte-macrophage stimulating factor; growth hormones--recombinant human; growth hormone--bovine; gentamycin; glucagon; glycopyrolate; gonadotropin releasing hormone and synthetic analogs thereof; GnRH; gonadorelin; grepafloxacin; haemophilus B conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus vaccine inactivated; heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3; insulin-human, insulin lispro; insulin procine; insulin NPH; insulin aspart; insulin glargine; insulin detemir; interferon alpha; interferon beta; ipratropium bromide; ifosfamide; Japanese encephalitis virus vaccine; lamivudine; leucovorin calcium; leuprolide acetate, levofloxacin; lincomycin and lincomycin derivatives; lobucavir; lomefloxacin; loracarbef; mannitol; is measles virus vaccine; meningococcal vaccine; menotropins; mepenzolate bromide; mesalamine; methenamine; methotrexate; methscopolamine; metformin hydrochloride; metoprolol; mezocillin sodium; mivacurium chloride; mumps viral vaccine; nedocromil sodium; neostigmine bromide; neostigmine methyl sulfate; neurontin; norfloxacin; octreotide

acetate; ofloxacin; olpadronate; oxytocin; pamidronate disodium; pancuronium bromide; paroxetine; perfloxacin; pentamidine isethionate; pentostatin; pentoxifylline; pericíclovir; pentagastrin; pentholamine mesylate; phenylalanine; physostigmine salicylate; plague vaccine; piperacillin sodium; platelet derived growth factor-human; pneumococcal vaccine polyvalent; poliovirus vaccine inactivated; poliovirus vaccine live (OPV); polymyxin B sulfate; pralidoxime chloride; pramlintide, pregabalin; propafenone; propenthaline bromide; pyridostigmine bromide; rabies vaccine; residronate; ribavarin; rimantadine hydrochloride; rotavirus vaccine; salmeterol xinafoate; sinealide; small pox vaccine; solatol; somatostatin; sparfloxacin; spectinomycin; stavudine; streptokinase; streptozocin; suxamethonium chloride; tacrine hydrochloride; terbutaline sulfate; thiopeta; ticarcillin; tiludronate; timolol; tissue type plasminogen activator; TNFR:Fc; TNK-tPA; trandolapril; trimetrexate gluconate; trospectinomycin; trovafloxacin; tubocurarine chloride; tumor necrosis factor; typhoid vaccine live; urea; urokinase; vancomycin; valacyclovir; valsartan; varicella virus vaccine live; vasopressin and vasopressin derivatives; vecuronium bromide; vinblastine; vincristine; vinorelbine; vitamin B12 ; warfarin sodium; yellow fever vaccine; zalcitabine; zanamivir; zolendronate; zidovudine; pharmaceutically acceptable salts, isomers and derivatives thereof; and mixtures thereof.

Detailed Description Text (19):
Various embodiments of the invention, as described in more detail below, include a hydrophilic surfactant. Hydrophilic surfactants can be used to provide any of several advantageous characteristics to the compositions, including: increased solubility of the active ingredient in the solid carrier; improved dissolution of the active ingredient; improved solubilization of the active ingredient upon dissolution; enhanced absorption and/or bioavailability of the active ingredient, particularly a hydrophilic active ingredient; and improved stability, both physical and chemical, of the active ingredient. The hydrophilic surfactant can be a single hydrophilic surfactant or a mixture of hydrophilic surfactants, and can be ionic or non-ionic.

Detailed Description Text (23):
It should be appreciated that the HLB value of a surfactant is merely a rough guide generally used to enable formulation of industrial, pharmaceutical and cosmetic emulsions. For many important surfactants, including several polyethoxylated surfactants, it has been reported that HLB values can differ by as much as about 8 HLB units, depending upon the empirical method chosen to determine the HLB value (Schott, J. Pharm. Sciences, 79(1), 87-88 (1990)). Likewise, for certain polypropylene oxide containing block copolymers (poloxamers, available commercially as PLURONIC.RTM. surfactants, BASF Corp.), the HLB values may not accurately reflect the true physical chemical nature of the compounds. Finally, commercial surfactant products are generally not pure compounds, but are often complex mixtures of compounds, and the HLB value reported for a particular compound may more accurately be characteristic of the commercial product of which the compound is a major component. Different commercial products having the same primary surfactant component can, and typically do, have different HLB values. In addition, a certain amount of lot-to-lot variability is expected even for a single commercial surfactant product. Keeping these inherent difficulties in mind, and using HLB values as a guide, one skilled in the art can readily identify surfactants having suitable hydrophilicity or lipophilicity for use in the present invention, as described herein.

Detailed Description Text (41):
In general, mixtures of surfactants are also suitable for use in the present invention. In particular, mixtures of propylene glycol fatty acid esters and glycerol fatty acid esters are suitable and are commercially available. Examples of these surfactants are shown in Table 8.

Detailed Description Text (65):
Ionizable surfactants, when present in their unionized (neutral, non-salt) form, are lipophilic surfactants suitable for use in the compositions of the present invention. Particular examples of such surfactants include free fatty acids, particularly C.sub.6 -C.sub.22 fatty acids, and bile acids. More specifically, suitable unionized ionizable surfactants include the free fatty acid and bile acid forms of any of the fatty acid salts and bile salts shown in Table 18.

Detailed Description Text (69):
Among the above-listed surfactants, several surfactants are preferred. In general, surfactants or mixtures of surfactants that solidify at ambient room temperature are most preferred. Also preferred are surfactants or mixtures of surfactants that solidify at ambient room temperature in combination with particular lipophilic components, such as triglycerides, or with addition of appropriate additives, such as viscosity modifiers, binders, thickeners, and the like.

Detailed Description Text (83):
For compositions of the present invention that include a lipophilic component, the lipophilic component can be a lipophilic surfactant or a triglyceride. Preferred triglycerides are those which solidify at ambient room temperature, with or without addition of appropriate additives, or those which in combination with particular surfactants and/or active ingredients solidify at room temperature. Examples of triglycerides suitable for use in the present invention are shown in Table 19. In general, these triglycerides are readily available from commercial sources. For several triglycerides, representative commercial products and/or commercial suppliers are listed.

Detailed Description Text (88):
The substrate of the compositions of the present invention can be a powder or a multiparticulate;, such as a granule, a pellet, a bead, a spherule, a beadlet, a microcapsule, a millisphere, a nanocapsule, a nanosphere, a microsphere, a platelet, platelet, a minitablet, a tablet or a capsule. A powder constitutes a finely divided (milled, micronized, nanosized, precipitated) form of an active ingredient or additive molecular aggregates or a compound aggregate of multiple components or a physical mixture of aggregates of an active ingredient and/or additives. Such substrates can be formed of various materials known in the art, such as, for example: Sugars, such as lactose, sucrose or dextrose; Polysaccharides, such as maltodextrin or dextrates; Starches; Cellulosics, such as microcrystalline cellulose or microcrystalline cellulose/sodium carboxymethyl cellulose; Inorganics, such as dicalcium phosphate, hydroxyapitite, tricalcium phosphate, talc, or titania; and Polyols, such as mannitol, xylitol, sorbitol or cyclodextrin.

Detailed Description Text (89):
The substrate can also be formed of any of the active ingredients, surfactants, triglycerides or additives described herein. In one particular embodiment, the substrate is a solid form of an additive, an active ingredient, a surfactant, or a triglyceride; a complex of an additive, surfactant or triglyceride and an active ingredient; a coprecipitate of an additive, surfactant or triglyceride and an active ingredient, or a mixture thereof.

Detailed Description Text (90):
It should be emphasized that the substrate need not be a solid material, although often it will be a solid. For example, the encapsulation coat on the substrate may act as a solid "shell" surrounding and encapsulating a liquid or semi-liquid substrate material. Such substrates are also within the scope of the present invention, as it is ultimately the carrier, of which the substrate is a part, which must be a solid.

Detailed Description Text (92):
The solid pharmaceutical compositions of the present invention can optionally

include one or more additives, sometimes referred to as excipients. The additives can be contained in an encapsulation coat in compositions which include an encapsulation coat, or can be part of the solid carrier, such as coated to an encapsulation coat, or contained within the components forming the solid carrier. Alternatively, the additives can be contained in the pharmaceutical composition but not part of the solid carrier itself. Specific, non-limiting examples of additives are described below.

Detailed Description Text (93):
Suitable additives are those commonly utilized to facilitate the processes involving the preparation of the solid carrier, the encapsulation coating, or the pharmaceutical dosage form. These processes include agglomeration, air suspension chilling, air suspension drying, balling, coacervation, comminution, compression, pelletization, cryopelletization, extrusion, granulation, homogenization, inclusion complexation, lyophilization, nanoencapsulation, melting, mixing, molding, pan coating, solvent dehydration, sonication, spheronization, spray chilling, spray congealing, spray drying, or other processes known in the art. The additive can also be pre-coated or encapsulated. Appropriate coatings are well known in the art, and are further described in the sections below. Based on the functionality of the additives, examples of the additives are as follows:

Detailed Description Text (95):
The pharmaceutical compositions of the present invention can optionally include one or more solubilizers, i.e., additives to increase the solubility of the pharmaceutical active ingredient or other composition components in the solid carrier. Suitable solubilizers for use in the compositions of the present invention include: alcohols and polyols, such as ethanol, isopropanol, butanol, benzyl alcohol, ethylene glycol, propylene glycol, butanediols and isomers thereof, glycerol, pentaerythritol, sorbitol, mannitol, transcutol, dimethyl isosorbide, polyethylene glycol, polypropylene glycol, polyvinylalcohol, hydroxypropyl methylcellulose and other cellulose derivatives, cyclodextrins and cyclodextrin derivatives; ethers of polyethylene glycols having an average molecular weight of about 200 to about 6000, such as tetrahydrofurfuryl alcohol PEG ether (glycofurol, available commercially from BASF under the trade name Tetraglycol) or methoxy PEG (Union Carbide); amides, such as 2-pyrrolidone, 2-piperidone, .epsilon.-caprolactam, N-alkylpyrrolidone, N-hydroxyalkylpyrrolidone, N-alkylpiperidone, N-alkylcaprolactam, dimethylacetamide, and polyvinylpyrrolidone; esters, such as ethyl propionate, tributylcitrate, acetyl triethylcitrate, acetyl tributyl citrate, triethylcitrate, ethyl oleate, ethyl caprylate, ethyl butyrate, triacetin, propylene glycol monoacetate, propylene glycol diacetate, .epsilon.-caprolactone and isomers thereof, .delta.-valerolactone and isomers thereof, .beta.-butyrolactone and isomers thereof; and other solubilizers known in the art, such as dimethyl acetamide, dimethyl isosorbide (Arlasolve DMI (ICI)), N-methyl pyrrolidones (Pharmasolve (ISP)), monooctanoin, diethylene glycol monoethyl ether (available from Gattefosse under the trade name Transcutol), and water.

Detailed Description Text (105):
Other additives conventionally used in pharmaceutical compositions can be included, and these additives are well known in the art. Such additives include: anti-adherents (anti-sticking agents, glidants, flow promoters, lubricants) such as talc, magnesium stearate, fumed silica (Carbosil, Aerosil), micronized silica (Syloid No. FP 244, Grace U.S.A.), polyethylene glycols, surfactants, waxes, stearic acid, stearic acid salts, stearic acid derivatives, starch, hydrogenated vegetable oils, sodium benzoate, sodium acetate, leucine, PEG-4000 and magnesium lauryl sulfate; anticoagulants, such as acetylated monoglycerides; antifoaming agents, such as long-chain alcohols and silicone derivatives; antioxidants, such as BHT, BHA, gallic acid, propyl gallate, ascorbic acid, ascorbyl palmitate, 4-hydroxymethyl-2,6-di-tert-butyl phenol, and tocopherol; binders (adhesives), i.e., agents that impart cohesive properties to powdered materials through particle-particle bonding, such as matrix binders (dry starch, dry sugars), film binders

(PVP; starch paste, celluloses, bentonite, sucrose), and chemical binders (polymeric (polymeric cellulose derivatives, such as carboxy methyl cellulose, HPC and HPMC; sugar syrups; corn syrup; water soluble polysaccharides such as acacia, tragacanth, guar and alginates; gelatin; gelatin hydrolysate; agar; sucrose; dextrose; and non-cellulosic binders, such as PVP, PEG, vinyl pyrrolidone copolymers, pregelatinized starch, sorbitol, and glucose); bufferants, where the acid is a pharmaceutically acceptable acid, such as hydrochloric acid, hydrobromic acid, hydriodic acid, sulfuric acid, nitric acid, boric acid, phosphoric acid, acetic acid, acrylic acid, adipic acid, alginic acid, alkanesulfonic acid, amino acids, ascorbic acid, benzoic acid, boric acid, butyric acid, carbonic acid, citric acid, fatty acids, formic acid, fumaric acid, gluconic acid, hydroquinosulfonic acid, isoascorbic acid, lactic lactic acid, maleic acid, methanesulfonic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid and uric acid, and where the base is a pharmaceutically acceptable base, such as an amino acid, an amino acid ester, ammonium hydroxide, potassium hydroxide, sodium hydroxide, sodium hydrogen carbonate, aluminum hydroxide, calcium carbonate, magnesium hydroxide, magnesium aluminum silicate, synthetic aluminum silicate, synthetic hydrotalcite, magnesium aluminum hydroxide, diisopropylethylamine, ethanolamine, ethylenediamine, triethanolamine, triethylamine, triisopropanolamine, or a salt of a pharmaceutically acceptable cation and acetic acid, acrylic acid, adipic acid, alginic acid, alkanesulfonic acid, an amino acid, ascorbic acid, benzoic acid, boric acid, butyric acid, carbonic carbonic acid, citric acid, a fatty acid, formic acid, fumaric acid, gluconic acid, hydroquinosulfonic acid, isoascorbic acid, lactic acid, maleic acid, methanesulfonic methanesulfonic acid, oxalic acid, para-bromophenylsulfonic acid, propionic acid, p-p-toluenesulfonic acid, salicylic acid, stearic acid, succinic acid, tannic acid, tartaric acid, thioglycolic acid, toluenesulfonic acid, and uric acid; chelating agents, such as EDTA and EDTA salts; coagulants, such as alginates; colorants or opaquants, such as titanium dioxide, food dyes, lakes, natural vegetable colorants, iron oxides, silicates, sulfates, magnesium hydroxide and aluminum hydroxide; coolants, such as halogenated hydrocarbons (e.g., trichloroethane, trichloroethylene, dichloromethane, fluorotrichloromethane), diethylether and liquid liquid nitrogen; cryoprotectants, such as trehelose, phosphates, citric acid, tartaric acid, gelatin, dextran and mannitol; diluents or fillers, such as lactose, mannitol, talc, magnesium stearate, sodium chloride, potassium chloride, citric acid, spray-dried lactose, hydrolyzed starches, directly compressible starch, microcrystalline cellulose, cellulosics, sorbitol, sucrose, sucrose-based materials, materials, calcium sulfate, dibasic calcium phosphate and dextrose, disintegrants or or super disintegrants, such as croscarmellose sodium, starch, starch derivatives, clays, gums, cellulose, cellulose derivatives, alginates, crosslinked polyvinypyrrolidone, sodium starch glycolate and microcrystalline cellulose; hydrogen bonding agents, such as magnesium oxide; flavorants or desensitizers, such as spray-dried flavors, essential oils and ethyl vanillin; ion-exchange resins, such such as styrene/divinyl benzene copolymers, and quaternary ammonium compounds; plasticizers, such as polyethylene glycol, citrate esters (e.g., triethyl citrate, acetyl triethyl citrate, acetyltributyl citrate), acetylated monoglycerides, glycerin, triacetin, propylene glycol, phthalate esters (e.g., diethyl phthalate, dibutyl phthalate), castor oil, sorbitol and dibutyl seccate; preservatives, such as as ascorbic acid, boric acid, sorbic acid, benzoic acid, and salts thereof, parabens, phenols, benzyl alcohol, and quaternary ammonium compounds; solvents, such such as alcohols, ketones, esters, chlorinated hydrocarbons and water; sweeteners, including natural sweeteners such as maltose, sucrose, glucose, sorbitol, glycerin and dextrins, and artificial sweeteners, such as aspartame, saccharine and saccharine salts; and thickeners (viscosity modifiers, thickening agents), such as sugars, polyvinylpyrrolidone, cellulosics, polymers and alginates.

Detailed Description Text (107):
It should be appreciated that there is considerable overlap between the above-listed additives in common usage, since a given additive is often classified

differently by different practitioners in the field, or is commonly used for any of several different functions. Thus, the above-listed additives should be taken as merely exemplary, and not limiting, of the types of additives that can be included in compositions of the present invention. The amounts of such additives can be readily determined by one skilled in the art, according to the particular properties properties desired.

Detailed Description Text (109):
The compositions of the present invention can be processed by agglomeration, air suspension chilling, air suspension drying, balling, coacervation, coating, comminution, compression, cryopelletization, encapsulation, extrusion, wet granulation, dry granulation, homogenization, inclusion complexation, lyophilization, melting, microencapsulation, mixing, molding, pan coating, solvent dehydration, sonication, spheronization, spray chilling, spray congealing, spray drying, or other processes known in the art. The compositions can be provided in the form of a minicapsule, a capsule, a tablet, an implant, a troche, a lozenge (minitablet), a temporary or permanent suspension, an ovule, a suppository, a wafer, a chewable tablet, a quick or fast dissolving tablet, an effervescent tablet, a buccal or sublingual solid, a granule, a film, a sprinkle, a pellet, a bead, a pill, a powder, a triturate, a platelet, a strip or a sachet. Compositions can also be administered as a "dry syrup", where the finished dosage form is placed directly on the tongue and swallowed or followed with a drink or beverage. These forms are well known in the art and are packaged appropriately. The compositions can be formulated for oral, nasal, buccal, ocular, urethral, transmucosal, vaginal, topical or rectal delivery, although oral delivery is presently preferred.

Detailed Description Text (110):
The pharmaceutical composition and/or the solid carrier particles can be coated with one or more enteric coatings, seal coatings, film coatings, barrier coatings, compress. coatings, fast disintegrating coatings, or enzyme degradable coatings. Multiple coatings can be applied for desired performance. Further, the dosage form can be designed for immediate release, pulsatile release, controlled release, extended release, delayed release, targeted release, synchronized release, or targeted delayed release. For release/absorption control, solid carriers can be made of various component types and levels or thicknesses of coats, with or without an active ingredient. Such diverse solid carriers can be blended in a dosage form to achieve a desired performance. The definitions of these terms are known to those skilled in the art. In addition, the dosage form release profile can be effected by a polymeric matrix composition, a coated matrix composition, a multiparticulate composition, a coated multiparticulate composition, an ion-exchange resin-based composition, an osmosis-based composition, or a biodegradable polymeric composition. Without wishing to be bound by theory, it is believed that the release may be effected through favorable diffusion, dissolution, erosion, ion-exchange, osmosis or combinations thereof.

Detailed Description Text (112):
Seal coating, or coating with isolation layers: Thin layers of up to 20 microns in thickness can be applied for variety of reasons, including for particle porosity reduction, to reduce dust, for chemical protection, to mask taste, to reduce odor, to minimize gastrointestinal irritation, etc. The isolating effect is proportional to the thickness of the coating. Water soluble cellulose ethers are preferred for this application. HPMC and ethyl cellulose in combination, or Eudragit E100, may be particularly suitable for taste masking applications. Traditional enteric coating materials listed elsewhere can also be applied to form an isolating layer.

Detailed Description Text (114):
Enteric coating; The term "enteric coating" as used herein relates to a mixture of pharmaceutically acceptable excipients which is applied to, combined with, mixed with or otherwise added to the carrier or composition. The coating may be applied to to a compressed or molded or extruded tablet, a gelatin capsule, and/or pellets,

beads, granules or particles of the carrier or composition. The coating may be applied through an aqueous dispersion or after dissolving in appropriate solvent. Additional additives and their levels, and selection of a primary coating material or materials will depend on the following properties: 1. resistance to dissolution and disintegration in the stomach; 2. impermeability to gastric fluids and drug/carrier/enzyme while in the stomach; 3. ability to dissolve or disintegrate rapidly at the target intestine site; 4. physical and chemical stability during storage; 5. non-toxicity; 6. easy application as a coating (substrate friendly); and 7. economical practicality.

Detailed Description Text (115):
Dosage forms of the compositions of the present invention can also be formulated as enteric coated delayed release oral dosage forms, i.e., as an oral dosage form of a pharmaceutical composition as described herein which utilizes an enteric coating to effect release in the lower gastrointestinal tract. The enteric coated dosage form may be a compressed or molded or extruded tablet/mold (coated or uncoated) containing granules, pellets, beads or particles of the active ingredient and/or other composition components, which are themselves coated or uncoated. The enteric coated oral dosage form may also be a capsule (coated or uncoated) containing pellets, beads or granules of the solid carrier or the composition, which are themselves coated or uncoated.

Detailed Description Text (119):
Cellulose Derivatives (also preferred). Examples of suitable cellulose derivatives are: ethyl cellulose; reaction mixtures of partial acetate esters of cellulose with phthalic anhydride. The performance can vary based on the degree and type of substitution. Cellulose acetate phthalate (CAP) dissolves in pH>6. Aquateric (FMC) is an aqueous based system and is a spray dried CAP psuedolatex with particles<;1 .mu.m. Other components in Aquateric can include pluronics, Tweens, and and acetylated monoglycerides; cellulose acetate trimellitate (Eastman); methylcellulose (Pharmacoat, Methocel); hydroxypropyl methyl cellulose phthalate (HPMCP). The performance can vary based on the degree and type of substitution. HP-50, HP-55, HP-55S, HP-55F grades are suitable; hydroxypropyl methyl cellulose succinate (HPMCS; AQOAT (Shin Etsu)).

Detailed Description Text (122):
The coating can, and usually does, contain a plasticizer and possibly other coating excipients such as colorants, talc, and/or magnesium stearate, which are well known in the art. Suitable plasticizers include: triethyl citrate (Citroflex 2), triacetin (glyceryl triacetate), acetyl triethyl citrate (Citroflec A2), Carbowax 400 (polyethylene glycol 400), diethyl phthalate, tributyl citrate, acetylated monoglycerides, glycerol, fatty acid esters, propylene glycol, and dibutyl phthalate. In particular, anionic carboxylic acrylic polymers usually will contain 10-25% by weight of a plasticizer, especially dibutyl phthalate, polyethylene glycol, triethyl citrate and triacetin. Conventional coating techniques such as spray or pan coating are employed to apply coatings. The coating thickness must be sufficient to ensure that the oral dosage form remains intact until the desired site of topical delivery in the lower intestinal tract is reached.

Detailed Description Text (125):
Another methacrylic acid polymer which is suitable for use in coating the composition or solid carrier which can be employed in the compositions and methods described herein, either alone or in combination with other coatings, is Eudragit S.RTM, manufactured by Rohm Pharma, Germany. Eudragit S.RTM. differs from Eudragit L-30-D.RTM only insofar as the ratio of free carboxyl groups to ester groups is approximately 1:2. Eudragit S.RTM is insoluble at pH below 5.5, but unlike Eudragit L-30-D.RTM, is poorly soluble in gastrointestinal fluids having pH of 5.5-7.0, such as is present in the small intestine media. This copolymer is soluble at pH 7.0 and above, i.e., the pH generally found in the colon. Eudragit S.RTM can be used alone as a coating to provide delivery of beginning at the large intestine via a delayed

release mechanism. In addition, Eudragit S.RTM, being poorly soluble in intestinal fluids below pH 7, can be used in combination with Eudragit L-30-D.RTM, soluble in intestinal fluids above pH 5.5, in order to effect a delayed release composition. The more Eudragit L-30 D. RTM used the more proximal realease and delivery begins, and the more Eudragit S.RTM used, the more distal release and delivery begins Both Eudragit L-30-D-RTM and Eudragit S.RTM can be substituted with other pharmaceutically acceptable polymers with similar pH solubility characteristics.

Detailed Description Text (128):
Fast-Disintegrating Coatings for Immediate Release: Immediate release coating of solid carriers is commonly used to improve product elegance as well as for a moisture barrier, and taste and odor masking. Rapid breakdown of the film in gastric media is important, leading to effective disintegration and dissolution. Eudragit RD100 (Rohm) is an example of such a coating. It is a combination of a water insoluble cationic methacrylate copolymer with a water soluble cellulose ether. In powder form, it is readily dispensible into an easily sprayable suspension that dries to leave a smooth film. Such films rapidly disintegrate in aqueous media at a rate that is independent of pH and film thickness.

Detailed Description Text (130):
The compositions of the present invention can be prepared by a variety of processes to apply an encapsulation coat onto a substrate or to form a substrate-free solid carrier such as a multiparticulate or a powder. The commonly utilized coating and pelletization processes include balling, spheronization, extrusion, spray congealing, spray drying, pan coating, fluidized bed coating, melt extrusion, crystallization, cryopelletization, nanoencapsulation, coacervation, etc. It is also clear to one skilled in the art that appropriate additives can also be introduced to the composition or during the processes to facilitate the preparation of the solid carrier or the dosage forms, depending on the need of the individual process.

Detailed Description Text (132):
A pelletization process typically involves preparing a molten solution of the composition of the solid carrier or a dispersion of the composition of the solid carrier solubilized or suspended in an aqueous medium, an organic solvent, a supercritical fluid, or a mixture thereof. Such solution or dispersion is then passed through a certain opening to achieve the desired shape, size, and other properties. Similarly, appropriate drying processes can be adopted to control the level of the residual dispersing medium, if necessary.

Detailed Description Text (135):
In a broad sense, pellets are very much like granules and bead; the techniques for producing pellets can also produce granules, beads, etc. Pellets, granules or beads are formed with the aid of a pelletizer, spheronizer or extruder. The pelletizer, spheronizer or extruder is able to form approximately spherical bodies from a mass of finely divided particles continuously, by a rolling or tumbling action on a flat or curved surface with the addition of a liquid.

Detailed Description Text (138):
Similarly, the choice of an appropriate binder for a given application is readily determined by one skilled in the art. At a minimum, the binder must be capable of wetting the surfaces of the particle being pelletized or granulated. Binders must have sufficient wet strength to allow agglomerates to be handled, and sufficient dry strength to make them suitable for their intended purposes. Each process, however, makes use of a different system of forces and may require a different agglomerate strength. The final selection of the binder should be made on the basis of the type of equipment that is used. The size and size distribution of pellets, bulk density, strength and flow properties also affect the performance of the pellets, and these properties can be adjusted by one skilled in the art by the inclusion of additives, choice of equipment, and processing conditions.

Detailed Description Text (140):
Extrusion is a well-known method of applying pressure to a damp or melted composition until it flows through an orifice or a defined opening. The extrudable length varies with the physical characteristics of the material to be extruded, the method of extrusion, and the process of manipulation of the particles after extrusion. Various types of extrusion devices can be employed, such as screw, sieve and basket, roll, and ram extruders.

Detailed Description Text (144):
Spheronization is the process of converting material into spheres, the shape with the lowest surface area to volume ratio. Spheronization typically begins with damp extruded particles. The extruded particles are broken into uniform lengths instantaneously and gradually transformed into spherical shapes. In addition, powdered raw materials, which require addition of either liquid or material from a mixer, can be processed in an air-assisted spheronizer.

Detailed Description Text (146):
Spray congealing is method that is generally used in changing the structure of the materials, to obtain free flowing powders from liquids and to provide pellets ranging in size from about 0.25 to 2.0 mm. Spray congealing is process in which a substance of interest is allowed to melt, disperse, or dissolve in a hot melt of other additives, and is then sprayed into an air chamber wherein the temperature is below the melting point of the formulation components, to provide spherical congealed pellets. The air removes the latent heat of fusion. The temperature of the cooled air used depends on the freezing point of the product. The particles are held together by solid bonds formed from the congealed melts. Due to the absence of solvent evaporation in most spray congealing processes, the particles are generally non porous and strong, and remain intact upon agitation. The characteristics of the final congealed product depend in part on the properties of the additives used. The rate of feeding and inlet/outlet temperatures are adjusted to ensure congealing of the atomized liquid droplet. The feed should have adequate viscosity to ensure homogeneity. The conversion of molten feed into powder is a single, continuous step. Proper atomization and a controlled cooling rate are critical to obtain high surface area, uniform and homogeneous congealed pellets. Adjustment of these parameters is readily achieved by one skilled in the art.

Detailed Description Text (148):
Conventional spray dryers operating with cool inlet air have been used for spray congealing. Several methods of atomization of molten mass can be employed, such as pressure, or pneumatic or centrifugal atomization. For persons skilled in the spray congealing art, it is well known that several formulation aspects, such as matrix materials, viscosity, and processing factors, such as temperature, atomization and cooling rate affect the quality (morphology, particle size distribution, polymophism and dissolution characteristics) of spray congealed pellets. The spray congealed particles may be used in tablet granulation form, encapsulation form, or can be incorporated into a liquid suspension form.

Detailed Description Text (150):
For compositions that are oily in nature, the spray drying technique is commonly employed. The oily material is commonly mixed with a polymeric material, such as gelatin, vegetable gum, modified starch, dextrin, or other appropriate additives. An emulsifier is added, if needed, to form an oil-in-water emulsion. The emulsion is atomized into a column of heated air in a drying chamber, resulting in rapid evaporation of water. Alternatively, the emulsion is atomized directly into a polar solvent, such as isopropanol, ethanol, glycerol or polyglycols, to dehydrate the aerosolized particle. This method is particularly suitable for compositions containing lipophilic actives or additives that result in lipophilic cores. Spray drying/solvent dehydration can also be applied to hydrophilic active ingredients or additives to form an oil in water emulsion which is spray dried. This results in a

homogenous solid composition. Furthermore, water or organic solvent based formulations can be spray dried by using inert process gas, such as nitrogen, argon and the like.

Detailed Description Text (158):
Coacervation is a transfer of macromolecules with film properties from a solvated state in a coacervation phase into a phase in which there is a film around each particle. The coacervation method involves dispersing the composition in a dispersion of a polymeric colloid, such as gelatin alginate, and shock treating the mixture with temperature or pH, etc., to generate a two-phase system. The desired phase is then hardened with a cross-linking agent, such as glutaraldehyde.

Detailed Description Text (160):
The cryopelletization procedure allows conversion of a molten mass, aqueous solution or suspension into solid, bead-like particles. The molten mass solutions or or suspensions are dripped by means of an appropriately designed device into liquid nitrogen. The production of small drops and liquid nitrogen cooling permit very rapid and uniform freezing of the material processed. The pellets are further dried in conventional freeze dryers. Cryopelletization can also be carried out under aseptic conditions for sterile processing. The most critical step producing spherical particles by globulization is the droplet formation. Droplet formation is influenced by formulation related variables, such as the nature of the active ingredient and additives, viscosity, total solid content, surface tension, etc. Extra care must be undertaken with processing of suspensions to ensure homogeneity. In addition, equipment design and processing variable also play an important role. One skilled in the art can readily balance the various factors to produce a satisfactory product. Enteric matrix pellets can be formed that include polyacrylic acid (e.g. Carbopol) with a high molecular weight polyethylene (such as PEG-20,000).

Detailed Description Text (162):
For processing of encapsulated compositions, various methods can be used. The term "microencapsulation" applies to enclosure or encasement in microcapsules. Microencapsulation is a means of applying coatings to small particles of solids or droplets of liquids and dispersions. The terms "coated", "protected" or "layered" are commonly used interchangeably with the term "encapsulated". All of these terms can be used to refer to practically any core material that is encased or enclosed in an outer shell. Typical equipment used to apply coating includes a conventional pan (Pellegrini; Italy), a modified perforated pan (multicoater, Thomas Eng., Ill.) or a Wurster coater in a Glatt powder doater/granulator (Glatt Airtechniques).

Detailed Description Text (165):
Substrate surface area, shape, porosity and stability are important determinants of good coating. Spherical particles are preferred, and these may be produced through spheronization or a spherical crystallization process. Crystals or compact granules from dry compaction or extrusion processes, often available commercially, serve as good substrates.

Detailed Description Text (169):
This process entails using coating materials that can be applied in a molten state. The selection of proper coating -materials depends on melting point, melting point range and the viscosity in the liquid state. A fluidized bed is ideal for molten coatings of substrates that range from about 100 microns to about 2000 microns in size. Fluidized bed coating, spraying molten materials, involves achieving a proper balance of process parameters that allow proper encapsulation to occur. Substrate particles that are suspended and separated from each other by the fluidization air enter a zone of finely atomized coating liquid. Coating occurs as the liquid droplets, which are substantially smaller in size than substrate, impact the particles, spread, and solidify. Multiple layers can be coated, and the completion of spraying is followed by a product stabilization or cooling step. Some critical

success parameters include bed temperature, atomization, atomization fluid temperature, or droplet size, spray type, spray rate, rate of coating droplet solidification on particle surfaces, particle size, shape, etc. Inert materials such such as sodium chloride, citric acid, potassium chloride can serve as substrates. One skilled in the art can readily adjust such parameters to achieve a satisfactory product.

Detailed Description Text (170):
The processes described above are suitable for treating substrate-based compositions or non-substrate-based compositions of the present invention. Thus, in one embodiment, pharmaceutical compositions of the present invention do not include a seed particle, such as a conventional drug or other additive aggregate starch or sugar bead. Instead, the compositions are processed, and the components are chosen, such that a solid composition is formed without the need to coat the composition onto a substrate bead. Such compositions can be in the form of beadlets, beads, granules, pellets, etc., that have an approximately homogenous distribution of active ingredient, surfactant, triglyceride and/or additives. These compositions can be produced by means of balling in pelletizers or fluid bed granulators, and compaction or extrusion/spheronization. In addition, these compositions can be produced using solvent-free spray congealing processes or dropping (globulization) methods. Dropping procedures involve conversion of aqueous solutions or suspensions to a solid form. Congealing of the liquid droplets in cooling baths can aided by a chemical reaction (e.g., insoluble salt or complex formation), a sol/gel transition, or by freezing in a coolant bath of liquid nitrogen or halogenated hydrocarbons.

Detailed Description Text (172):
In one embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier including a substrate and an encapsulation coat on the substrate. The encapsulation coat includes at least one ionic or non-ionic hydrophilic surfactant. Optionally, the encapsulation coat can include a pharmaceutical active ingredient, a lipophilic component such as a lipophilic surfactant or a triglyceride, or both a pharmaceutical active ingredient and a lipophilic component.

Detailed Description Text (173):
Prior art has used surfactants in formulating coated bead compositions to provide a wetting function, to enable hydrophobic drugs to properly adhere to beads and/or water-soluble binders. For example, U.S. Pat. No. 4,717,569 to Harrison et al. discloses coated bead compositions of hydrophobic steroid compounds wetted by a hydrophilic surfactant and adhered to the beads by a water-soluble binder. The steroid compound is present as finely divided particles, held to the beads by the binder. The present inventors have surprisingly found that proper choice of surfactants and other components allows compositions to be prepared with a wide variety of hydrophilic or hydrophobic active ingredients. For example, while the Harrison reference discloses the use of surfactants as wetting agents, the present inventors have found that surfactants at higher levels, i.e., in amounts far in excess of the amounts necessary or appropriate for a wetting function, enable a pharmaceutical active ingredient to be fully or at least partially solubilized in the encapsulation coating material itself, rather than merely physically bound in a binder matrix. In fact, while binders can optionally be used in the compositions of the present invention, the higher surfactant concentrations of the present invention, i.e., solubilizing amounts, obviate the need for binders and render them optional instead of necessary.

Detailed Description Text (179):
In another embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier, including a substrate and an encapsulation coat on the substrate. The encapsulation coat includes a lipophilic component, such as a lipophilic surfactant or a triglyceride. Optionally, the encapsulation coat can

include a pharmaceutical active ingredient, an ionic or non-ionic hydrophilic surfactant, or both a pharmaceutical active ingredient and a hydrophilic surfactant. surfactant. In this embodiment, the lipophilic surfactant or triglyceride can be present in amounts to enable at least partial solubilization of an active ingredient ingredient in the encapsulation coat, in the composition, or separately administered.

Detailed Description Text (181):
In another embodiment, the solid pharmaceutical composition includes a solid carrier, the solid carrier including a substrate and an encapsulation coat on the substrate. The encapsulation coat includes a pharmaceutical active ingredient and an ionic or non-ionic hydrophilic surfactant; a pharmaceutical active ingredient and a lipophilic component such as a lipophilic surfactant or a triglyceride; or a pharmaceutical active ingredient and both a hydrophilic surfactant and a lipophilic component.

Detailed Description Text (182):
In another embodiment, the solid pharmaceutical composition includes a solid, carrier, wherein the solid carrier is formed of at least two components selected from the group consisting of pharmaceutical active ingredients; ionic or non-ionic hydrophilic surfactants; and lipophilic components such as lipophilic surfactants and triglycerides.

Detailed Description Text (183):
In this embodiment, the solid pharmaceutical composition is formulated without the need for a substrate seed particle. The active ingredient, surfactants and triglycerides in the chosen combination are processed, with appropriate excipients if necessary, to form solid carriers in the absence of a seed substrate. Preferably, Preferably, the components are chosen to at least partially solubilize the active ingredient, as described above.

Detailed Description Text (189):
In another aspect of the invention, the solid carrier improves the chemical stability of the active ingredient.

Detailed Description Text (190):
In another aspect of the invention, the solid carrier protects the upper gastrointestinal tract from the adverse effects of the active ingredient.

Detailed Description Text (194):
Preparation of Coated Beads

Detailed Description Text (197):
Commercially available sugar beads (30/35 mesh size) were coated in a conventional coating pan having a spray gun (Campbell Hausfield, DH 7500) with a nozzle diameter of 1.2 mm and an air pressure of 25 psi. The bed temperature was maintained at approximately 32.degree. C. during the spraying process. Appropriate amounts of talc were sprinkled on the beads during the spraying process to reduce the agglomeration of coated beads. When the spraying process was completed, the coated beads were allowed to cool to room temperature. The coated beads were then dried under vacuum to minimize residual solvent levels. The dried, coated beads were then sieved and collected.

Detailed Description Text (200):
A pharmaceutical composition was prepared according to the method of Example 1, having a substrate particle, an active ingredient (glyburide), and a mixture of a hydrophilic surfactant (PEG-40 stearate) and a lipophilic surfactant (glycerol monolaurate). The components and their amounts were as follows:

Detailed Description Text (203):

A pharmaceutical composition was prepared according to the method of Example 1, having a substrate particle, an active ingredient (progesterone), a mixture of a hydrophilic surfactant (Solulan C-24) and two lipophilic components (deoxycholic acid and distilled monoglycerides). The components and their amounts were as follows:

Detailed Description Text (206):
A pharmaceutical composition was prepared according to the method of Example 1, having a substrate particle, an active ingredient (itraconazole), a mixture of non-ionic hydrophilic surfactants (Cremophor RH-40 and PEG-150 monostearate), an ionic hydrophilic surfactant (sodium taurocholate) and a lipophilic surfactant (glycerol monolaurate). The components and their amounts were as follows:

Detailed Description Text (209):
A pharmaceutical composition was prepared according to the method of Example 1, having a substrate particle, an active ingredient (omeprazole), a mixture of a two hydrophilic surfactants (PEG-150 monostearate and PEG-40 monostearate), and a triglyceride-containing lipophilic component (Maisine 35-1). The components and their amounts were as follows:

Detailed Description Text (212):
The dried, coated beads of Example 3 were further seal coated by a polymer layer. The seal coating polymer layer was applied to the progesterone-coated beads in a Uni-Glatt fluid bed coater. Polyvinylpyrrolidone (PVP-K30) was dissolved in isopropyl alcohol to form a 5% w/w solution. This seal coating solution was sprayed onto the coated beads with a Wurster bottom spray insert. The inlet and outlet air temperature were maintained at 30 and 40.degree. C., respectively. When the spraying process was complete, the beads were further dried by supplying dry air at 50-55.degree. C. for 5-15 minutes. The seal coated beads were then allowed to cool in the apparatus by supplying dry air at 20-25.degree. C. for 5-15 minutes. The dried, seal coated beads were collected and stored in a container.

Detailed Description Text (215):
The dried, coated beads of Example 5 were further coated with a protective polymer layer. The protective coating was applied to the omeprazole coated beads by spraying with a hydroxypropyl methylcellulose (HPMC) solution. The protective coating HPMC solution was prepared by dissolving 6 grams of HPMC in ethanol. To this solution, methylene chloride was added followed by 2 mL of triethylcitrate as a plasticizer and 1 g of talc the HPMC solution was sprayed on the beads as described in Example 6, and the protective coated beads were then dried and collected.

Detailed Description Text (218):
The dried, coated beads of Example 5 were further coated with an enteric coating layer. The enteric layer was applied to the omeprazole coated beads by spraying a Eudragit L100 solution. Eudragit L100 is an acrylate polymer commercially available from Rohm Pharma. The spraying solution was prepared by dispersing 15 g of Eudragit L100 in 200 mL of ethanol to give a clear solution. To this solution, 4 g of triethyl citrate was then added as a plasticizer. 2 grams of purified talc was also added to the solution. The solution was then sprayed onto the beads, and the beads were dried, as described in Example 6.

Detailed Description Text (221):
A comparative dissolution study was performed on three forms of an active ingredient: the glyburide coated beads of Example 2, a commercially available glyburide product (Micronase.RTM., available from Pharmacia & Upjohn), and the pure glyburide bulk drug. The dissolution study was performed using a USP dissolution type 2 apparatus. For each of the three forms, material equivalent to 5 mg of glyburide was used for each triplicated dissolution run in 500 mL of isotonic pH 7.4 phosphate buffer. The dissolution medium was maintained at 37.degree. C. and

constantly stirred at a speed of 100 rpm. The dissolution media were sampled at 15, 30, 45, 60, 120 and 180 minutes. At each time point, 3 mL of the medium was sampled, sampled, and the medium was replenished with 3 mL of fresh buffer. The samples were filtered through a 0.45.mu. filter immediately after the sampling. The filtrates were then diluted in methanol to an appropriate concentration for a glyburide-specific HPLC assay.

Detailed Description Text (223):
The results of the comparative dissolution measurement were expressed as the percent of glyburide dissolved/released in the dissolution medium at a given time, relative to the initial total glyburide content present in the dissolution medium (5 mg/500 mL). The results are shown in FIG. 1, with the error bars representing the standard deviation. As the Figure shows, the glyburide coated beads of the present invention showed a superior dissolution profile in the rate, the extent, and the variability of glyburide dissolved/released into the dissolution medium, compared to the commercial Micronase.RTM. and the pure bulk drug.

Detailed Description Text (226):
A comparative dissolution study was performed on three forms of an active ingredient: the progesterone coated beads of Example 3, the seal coated, progesterone coated beads of Example 6, and the pure progesterone bulk drug. The dissolution study was performed using a USP dissolution type 2 apparatus. For each of the three forms, material equivalent to 100 mg of progesterone was used for each duplicated dissolution run in 900 mL of isotonic pH 7.4 phosphate buffer containing 0.5% w/v of Tween 80. The dissolution medium was maintained at 37.degree. C. and constantly stirred at a speed of 100 rpm. The dissolution media were sampled at 30, 60, 120 and 180 minutes. At each time point, 3 mL of the medium was sampled, and the medium was replenished with 3 mL of fresh buffer/Tween solution. The samples were filtered through a 0.45.mu. filter immediately after the sampling. The filtrates were then diluted in methanol to an appropriate concentration for a progesterone-specific HPLC assay.

Detailed Description Text (228):
The results of the comparative dissolution measurement were expressed as the percent of progesterone dissolved/released in the dissolution medium at a given time, relative to the initial total progesterone content present in the dissolution medium (100 mg/900 mL). The results are shown in FIG. 2A. As the Figure shows, the progesterone coated beads of the present invention, with or without a seal coating, showed superior dissolution profiles in both the rate and the extent of progesterone dissolved/released into the dissolution medium, compared to the pure bulk drug.

Detailed Description Text (231):
A comparative dissolution study was performed on three forms of an active ingredient: the progesterone coated beads of Example 3, the seal coated, progesterone coated beads of Example 6, and the pure progesterone bulk drug. Prometrium.RTM. is a capsule dosage form in which micronized progesterone is suspended in a blend of vegetable oils. The dissolution of the Prometrium.RTM. capsule was performed using a USP dissolution type 1 apparatus, and the dissolution of the other samples was performed using a USP dissolution type 2 apparatus. For each of the three forms, material equivalent to 100 mg of progesterone was used for each dissolution run in 900 mL of isotonic pH 7.4 phosphate buffer. The dissolution medium was maintained at 37.degree. C. and constantly stirred at a speed of 100 rpm. The dissolution media were sampled at 15, 30, 45, 60 and 180 minutes. At each time point, 3 mL of the medium was sampled, and the medium was replenished with 3 mL of fresh buffer/Tween solution. The samples were filtered through a 0.45.mu. filter immediately after the sampling. The filtrates were then diluted in methanol to an appropriate concentration for a progesterone-specific HPLC assay.

Detailed Description Text (233):

The results of the comparative dissolution measurement were expressed as the percent percent of progesterone dissolved/released in the dissolution medium at a given time, relative to the initial total progesterone content present in the dissolution medium (100 mg/900 mL). The results are shown in FIG. 2B. As the Figure shows, the progesterone coated beads of the present invention, with or without a seal coating, showed superior dissolution profiles in both the rate and the extent of progesterone dissolved/released into the dissolution medium, compared to the commercial Prometrium.RTM. and the pure bulk drug.

Detailed Description Text (236):
A comparative dissolution study was performed comparing the rate and extent of dissolution of the protective coated, omeprazole coated beads of Example 7, the enteric coated, omeprazole coated beads of Example 8 and a commercially available omeprazole product (Prilosec.RTM., available from Astra Zeneca). The dissolution study was performed using a USP dissolution type 2 apparatus. For each of the three dosage forms, material equivalent to 5 mg of omeprazole was used for each dissolution run in 500 mL of isotonic pH 7.4 phosphate buffer. The dissolution medium was maintained at 37.degree. C. and constantly stirred at a speed of 100 rpm. The dissolution media were sampled at 15, 30, 45 and 60 minutes. At each time point, 3 mL of the medium was sampled, and the medium was replenished with 3 mL of fresh buffer. The samples were filtered through a 0.45.mu. filter immediately after the sampling. The filtrates were then diluted in methanol to an appropriate concentration for an omeprazole-specific HPLC assay.

Detailed Description Text (238):
The results of the comparative dissolution measurement were expressed as the percent of omeprazole dissolved in the dissolution medium at a given time, relative to the initial total omeprazole content present in the dissolution medium (5 mg/500 mL). The results are shown in FIG. 3. As the Figure shows, the omeprazole coated beads of the present invention showed superior dissolution profiles in both the rate rate and the extent of omeprazole dissolved/released into the dissolution medium, compared to the commercial Prilose.RTM. product.

CLAIMS:

1. A pharmaceutical composition in the form of a solid carrier comprising a substrate and an encapsulation coat on the substrate, wherein the encapsulation coat comprises an admixture of a therapeutically effective amount of a hydrophobic pharmaceutical active ingredient, an effective solubilizing amount of at least one hydrophilic surfactant, and a lipophilic additive selected from the group consisting of lipophilic surfactants, triglycerides, and combinations thereof, wherein the effective solubilizing amount of the at least one hydrophilic surfactant is an amount effective to partially or fully solubilize the pharmaceutical active ingredient in the encapsulation coat.

16. The pharmaceutical composition of claim 13, wherein the substrate is a multiparticulate selected from the group consisting of a granule, a pellet, a bead, a spherule, a beadlet, a microcapsule, a millisphere, a nanocapsule, a nanosphere, a microsphere, a platelet, a tablet and a capsule.

17. The pharmaceutical composition of claim 1, wherein the solid carrier is a bead, a beadlet, a granule, a spherule, a pellet, a microcapsule, a microsphere, a nanosphere, a film, a wafer, a sprinkle, an implant, a troche, a lozenge, a platelet, a nanocapsule or a strip.

21. The pharmaceutical composition of claim 1, wherein the solid carrier is enteric coated, coated for fast disintegration, seal coated, film coated, barrier coated, compress coated, or coated with an enzyme-degradable coating.

23. The pharmaceutical composition of claim 1, in the form of a capsule, a tablet,

an ovule, a suppository, a wafer, a chewable tablet, a buccal tablet, a sub lingual tablet, a quick-dissolve tablet, an effervescent tablet, a granule, a pellet, a bead, a pill, a sachet, a sprinkle, a film, a dry syrup, a reconstitutable solid, a suspension, a lozenge, a troche, an implant, a power, a triturate, a platelet, or a strip.

25. The pharmaceutical composition of claim 1, wherein the composition is formulated for oral, nasal, ocular, urethral, buccal, transmucosal, vaginal, topical or rectal delivery.

29. A pharmaceutical composition in the form of a solid carrier comprising an admixture of a hydrophobic pharmaceutical active ingredient an effective solubilizing amount of at least one hydrophilic surfactant, and a lipophilic additive selected from the group consisting of lipophilic surfactants, triglycerides, and combinations thereof, wherein the effective solubilizing amount of the at least one hydrophilic surfactant is an amount effective to partially or fully solubilize the pharmaceutical active ingredient in the solid carrier.

42. The pharmaceutical composition of claim 29, wherein the solid carrier is a bead, a beadlet, a granule, a spherule, a pellet, a microcapsule, a microsphere, a nanosphere, a film, a wafer, a sprinkle, an implant, a troche, a lozenge, a platelet, a nanocapsule or a strip.

47. The pharmaceutical composition of claim 29, wherein the solid carrier is enteric coated, coated for fast disintegration, seal coated, film coated, barrier coated, compress coated, or coated with an enzyme-degradable coating.

49. The pharmaceutical composition of claim 29 in the form of a capsule, a tablet, an ovule, a suppository, a wafer, a chewable tablet, a buccal tablet, a sub-lingual tablet, a quick-dissolve tablet, an effervescent tablet, a granule, a pellet, a bead, a pill, a sachet, a sprinkle, a film, a dry syrup, a reconstitutable solid, a suspension, a lozenge, a troche, an implant, a powder, a triturate, a platelet, or a strip.

51. The pharmaceutical composition of claim 29, wherein the composition is formulated for oral, nasal, ocular, urethral, buccal, transmucosal, vaginal, topical or rectal delivery.

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L6: Entry 15 of 23 File: USPT Oct 15, 1996

DOCUMENT-IDENTIFIER: US 5565334 A
TITLE: Enhancer sequence for modulating expression in epithelial cells

CLAIMS:

23. The DNA of claim 22, wherein said DF3 enhancer or allelic variant is operatively operatively linked to a sequence encoding a heterologous polypeptide comprising an enzymatically active subunit of Pseudomonas exotoxin A, diphtheria toxin, Shiga toxin, Shiga-like toxin, E. coli LT, C3 toxin, pertuseis toxin, tetanus toxin, cholera toxin, or botulism toxin; gelonin; ricin; tumor necrosis factor; cAMP receptor polypeptide kinass; platelet factor 4; monocyte chemoattractants; herpes virus thymidine kinass; cytidine deaminase; WTp53; retinoblastoma protein; E-cadherin; fibronectin receptor; interleukin-2; interleukin-4; phenylalanine hydroxylass; cystic fibrosis transmembrane regulator; Factor VIII; Factor IX; or alpha.sub.1 antitrypsin; provided that said DF3 enhancer or allelic variant functions to increase tissue-specific expression of said sequence encoding said heterologous polypeptide in epithelial cells.

25. Isolated DNA comprising DF3 enhancer, said DF3 enhancer comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 8, said DF3 enhancer Being operatively linked to a sequence encoding a heterologous polypeptide comprising an enzymatically active subunit of Pseudomonas exotoxin A, diphtheria toxin, Shiga toxin, Shiga-like toxin, E. coli LT, C3 toxin, pertussis toxin, tetanus toxin, cholera toxin, or botulism toxin; gelonin; ricin; tumor necrosis factor; cAMP receptor polypeptide kinass; platelet factor 4; monocyte chemoattractants; herpes virus thymidine kinass; cytidine deaminase; WTp53; retinoblastoma protein; E-cadherin; fibronectin receptor; interleukin-2; interleukin-4; phenylalanine hydroxylase; cystic fibrosis transmembrane regulator; Factor VIII; Factor IX; or alpha.sub.1 antitrypsin; provided that said DF3 enhancer increases tissue-specific expression of said coding sequence in epithelial cells.

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↑ mammalian expression

no coding?

USPat 5,416,017

CLAIMS:

1. An isolated nucleic acid molecule comprising a tissue specific promoter operatively linked to a non-lethal modulator gene, wherein said non-lethal modulator gene is a cholera toxin gene, and wherein said tissue specific promoter is selected from the group consisting of a growth hormone promoter, an insulin promoter, and a neuron-specific enolase promoter.

2. The nucleic acid molecule of claim 1 wherein said tissue specific promoter is an insulin promoter.

3. The nucleic acid molecule of claim 1, wherein said promoter is a neuron-specific enolase promoter.

4. A cell containing the nucleic acid molecule of claim 1.

5. A vector containing the nucleic acid molecule of claim 1.

6. The vector of claim 5, wherein said vector is a virus.

7. The vector of claim 6, wherein said virus is selected from the group consisting of HSV, AAV, and adenovirus.

8. The vector of claim 5, wherein said vector is a retrovirus.

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DOCUMENT-IDENTIFIER: US 5416017 A
TITLE: Cholera toxin gene regulated by tissue-specific promoters

Brief Summary Text (5):
The cyclic nucleotides cyclic adenine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP) act as second messengers in many cell types to couple extracellular stimulatory or inhibitory signals with the appropriate cellular physiological responses. Examples of important cellular physiological responses that are regulated by cyclic nucleotide second messengers include visual excitation, activation of adenylate cyclase and growth hormone secretion by growth hormone releasing factor, regulation of ion channels by neurotransmitters and membrane receptor activation and olfaction. See, for example, Gilman, Ann. Rev. Biochem., 56:615-49 (1987); Neer et al., Nature, 333:129-134 (1988); Vallar et al., Nature, 330:566-568 (1987); Dolphin, Trends in Neurosciences, Volume 10, February (1987); and Stryer, Ann. Rev. Neurosci., 9:87-119 (1986).

Brief Summary Text (6):
The hormone-responsive tissues are the most extensively studied examples of regulation of physiological responses by cyclic nucleotides. For example, growth hormone releasing factor (GRF) is produced by the hypothalamus, and binds to a receptor on growth hormone (GH)-producing pituitary somatotrope cells. This binding causes stimulation of the ubiquitous receptor-associated G-protein G.sub.s, leading to activation of adenylate cyclase which in turn causes the elevation of cAMP levels within the somatotrope cells. Bilezikjian et al., Endocrinology, 113:1726-1731 (1983) and Vallar et al., Nature, 330:566-568 (1987). The elevated cAMP levels within the somatotrope cells increases the rate of growth hormone gene transcription and consequently the secretion of growth hormone from the somatotrope cells. Schofield, Nature, 215:1382-1383 (1967) and Barinaga et al., Nature, 314:279-281 (1985). The somatotrope cells also proliferate in response to the elevated cAMP level. Billesterup et al., Proc. Natl. Acad. Sci., USA, 83:6854-6857 (1986). Other G-protein dependent cascades with different specific stimulatory or inhibitory outcomes are found in other hormone-responsive tissues such as the adrenal cortex, thyroid and gonads, as well as in various subsets of neurotransmitter- or light-responsive neurons such as the retinal photoreceptors.

Brief Summary Text (13):
The method of the present invention for genetically programming a cell includes the steps of introducing into the cell a nucleic acid molecule comprised of a tissue specific promoter operatively linked to (controlling the expression of) a non-lethal modulator gene. The cell is then maintained so the non-lethal modulator gene present on the nucleic acid molecule can be expressed and genetically program the cell.

Brief Summary Text (14):
The present invention also contemplates an isolated nucleic acid comprising a tissue specific promoter controlling the expression of a non-lethal modulator gene and a non-lethal gene under the control of the tissue specific promoter. In preferred embodiments, the non-lethal modulator gene is a non-lethal second messenger system modulator gene or a non-lethal cyclic AMP modulator gene. In other preferred embodiments the non-lethal modulator is a cholera toxin gene or a pertussis toxin gene.

Brief Summary Text (15):
The present invention also contemplates cells, tissues, mammals, and plants containing a nucleic acid molecule of the present invention.

Drawing Description Text (2):
FIG. 1A-1F. The nucleotide sequence of cholera toxin as determined by Mekalanos et al., Nature, 306:551 (1983) is shown.

Drawing Description Text (3):
FIG. 2A-2D. In FIG. 2 the nucleotide sequence of pertussis toxin as determined by Locht et al., Science, 232:1558 is shown.

Drawing Description Text (4):
FIG. 3. The details of the construction of the nucleic acid molecule containing the tissue specific rat growth hormone promoter located 5' of and controlling the expression of the cholera toxin A1 subunit gene are shown. The upper portion of the figure shows the amino acid and nucleotide sequence of the portion of the cholera toxin (ctx) operon which encodes the mature and enzymatically active intracellular subunit of cholera toxin, A1 described by Mekalanos et at., Nature, 306:551-557 (1983), aligned with the nucleotide and predicted amino acid sequence of a PCR-amplified mouse CT cassette. The eukaryotic ATG initiation codon and TAG termination codon in the CT cassette are shown in boldface. The inclusion of the eukaryotic translation initiator, "CCACCATGG" described by Kozak, Nucleic Acid Research, 12:857:872 (1984) changes the predicted amino acid sequence of the mouse cholera toxin to add two N-terminal amino acids residues to the ctx-A1 subunit amino acid sequence. The Bam HI restriction endonuclease sites used for placing the cholera toxin cassette under the control of a tissue specific promoter are underlined. The PCR primers used to create the cassette were CT-5 (5' GCCCGGATCCACCATGGGTAATGATGATAAGTTATTTTAT 3') and CT-3 (5' GCGGATCCTACGATGATCTTGGAGCATTCC 3').

No Back Sigma peptide

Drawing Description Text (5):
The middle portion of the figure shows the construction of the nucleic acid molecule containing rGH-CT fusion gene by ligation of the 599 bp CT cassette into the BamHI site at position +13 within the 5' untranslated region of the rat growth hormone/human growth hormone (rGH-hGH) fusion gene that have been previously described by Behringer et al., Genes and Development, 2:453-461 (1988). The components of the rGH-hGH fusion gene are: shaded box (rGH promoter and 5' untranslated region), open boxes (hGH 5' untranslated region, introns, and 3' untranslated region) and solid boxes (hGH exons). Insertion of the cassette converts all human sequences into 3' untranslated sequence. The DNA sequence of the inserted CT cassette and flanking regions within rGH-CT was obtained to confirm error-free PCR amplification and cloning. The sequence of the 5+ untranslated region is shown at bottom. The complete rGH-CT structural gene is carried on a 3 kb KpnI-EcoRI fragment, which was excised from plasmid pUC and used for microinjection.

Drawing Description Text (6):
FIG. 4A-4L. The pituitary and somatotrope hyperplasia observed in transgenic mice expressing a recombinant nucleic acid molecule containing the tissue specific rat growth hormone promoter controlling the expression of the cholera toxin A1 subunit gene is shown. In FIG. 4A, whole pituitaries of control (left) and transgenic (right) mice. The lesion on the transgenic pituitary was diagnosed as a non-cancerous infarction presumably due to organ compression caused by anterior lobe expansion. In FIGS. 4B-4L, the immunohistocytochemical localization of cholera toxin (CT), growth hormone (GH), prolactin (PRL) and thyroid stimulating hormone (TSH) is shown. The pituitaries from transgenic mice expressing the cholera toxin A1 subunit are shown in FIGS. 4C-4G, 4I, 4J and 4L. The pituitaries from control mice are shown in FIGS. 4B, 4H, and 4K.

Drawing Description Text (10):
FIG. 5A-5D. FIGS. 5A-5D illustrate various transgenes prepared according to the present invention. In FIG. 5A, a neuron-specific enolase-cholera toxin (NSE-CT) transgene is shown. The CT open reading frame (ORF) corresponds to the A1 region of the cholera holotoxin operon, ctx. In FIG. 5B, the RV-LacZ-CT retroviral transgene is shown. A Moloney Murine-Leukemia Virus (Mo-MuLV) vector, which retains the viral long terminal repeats, but from which the gag-pol-env viral sequences have been

removed, was used to express both .beta.-galactosidase and cholera toxin from the LacZ and CT open reading frames, respectively.

Detailed Description Text (3):
A nucleic acid molecule of the present invention comprises a tissue specific promoter located 5+ of and controlling the expression of a non-lethal modulator gene. The tissue specific promoter is a DNA segment that contains a DNA sequence that control the expression of a gene located 3' or downstream of the promoter. The promoter is the DNA sequence to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene, typically located 3' of the promoter.

Detailed Description Text (4):
The subject nucleic acid molecule comprises at least 2 different operatively linked DNA segments. The DNA can be manipulated and amplified using the standard techniques described in Molecular Cloning: A Laboratory Manual, 2nd Edition, Maniatis et al., eds., Cold Spring Harbor, N.Y. (1989). Typically, to produce a nucleic acid molecule of the present invention, the tissue specific promoter and the non-lethal modulator gene are operatively linked to a vector DNA molecule capable of autonomous replication in a cell. By operatively linking the tissue specific promoter and the non-lethal modulator gene to the vector the attached segments are replicated along with the vector sequences. Thus, a recombinant DNA molecule (rDNA) is a hybrid DNA molecule comprising at least 2 nucleotide sequences not normally found together in nature.

Detailed Description Text (5):
In preferred embodiments, the recombinant nucleic acid molecule that is introduced into the cells of a mammal contains a tissue specific promoter and a non-lethal modulator gene and has had the excess vector sequences removed. These excess vector sequences are removed by cutting the recombinant nucleic acid molecule with 1 or more restriction endonucleases to produce a linear recombinant nucleic acid molecule containing a tissue specific promoter and a non-lethal modulator gene.

Detailed Description Text (6):
The nucleic acid molecule of the present invention is from about 100 base pairs to about 100,000 base pairs in length. Preferably the nucleic acid molecule is from about 50 base pairs to about 50,000 base pairs in length. More preferably the nucleic acid molecule is from about 50 base pairs to about 10,000 base pairs in length. Most preferred is a nucleic acid molecule from about 50 pairs to about 4,000 base pairs in length.

Detailed Description Text (9):
A recombinant nucleic acid molecule of the present invention comprises a non-lethal modulator gene located 3' of the tissue specific promoter. A non-lethal modulator gene of the present invention is a gene that codes for an intracellular modulator that alters a complex biochemical pathway of the cell in which the gene is expressed but is not lethal to that cell. The intracellular modulator of the present invention includes proteins, RNAs and DNAs that are made within a cell and modulate a complex biochemical activity of that same cell by directly altering a biochemical substance or process without first being excreted from that cell. The non-lethal modulator gene is expressed from the tissue-specific promoter located 5' of it and it directly modulates, that is increases or decreases the amount or activity of a biochemical substance or process.

Detailed Description Text (11):
In other preferred embodiments, the recombinant nucleic acid molecule of the present invention comprises a tissue specific promoter located 5' of a non-lethal modulator gene and a non-lethal second messenger system modulator gene. A non-lethal second messenger system modulator gene is a gene that codes for an intracellular modulator that alters a second messenger system of the cell in which the gene

is expressed but is not lethal to that cell. In addition, the non-lethal second messenger system modulator gene is not a homolog of a cellular gene within the cell it is introduced into. Examples of homologs of cellular genes include the oncogenes such as fos, myc, neu, and ras. Toxin genes such as the cholera toxin gene, the pertussis toxin gene and the E. coli heat-labile toxin gene do not have cellular homologs in eukaryotic cells.

Detailed Description Text (14):
In other preferred embodiments a recombinant nucleic acid molecule of the present invention comprises a tissue specific promoter controlling the expression of a non-lethal cholera toxin gene and a cholera toxin gene located 3' of the tissue specific promoter. For example, a non-lethal cholera toxin gene includes the sequence of the cholera toxin gene encoding the A1 polypeptide has been described by Mekalanos et al., Nature, 306:551-557 (1983). The present invention also contemplates a segment of the cholera toxin A1 gene that encode a polypeptide that is an enzyme that is capable of catalyzing the ADP-ribosylation and activation of G.sub.s, the GTP-binding regulatory component that stimulates the adenylate cyclase complex. A cholera toxin gene of the present invention includes those nucleic acid segments that non-randomly hybridize to nucleotides 569 to 1150 of FIG. 1 or to a complementary strand of nucleotides 569 to 1150 of FIG. 1.

Detailed Description Text (15):
In other preferred embodiments, a recombinant nucleic acid molecule of the present invention comprises a tissue specific promoter located 5' of a non-lethal pertussis toxin gene and a non-lethal pertussis toxin gene located 3' of the tissue specific promoter. A non-lethal pertussis toxin gene of the present invention includes the pertussis toxin genes described by Nicosia et al., Proc. Natl. Acad. Sci., 83:4631-4635 (1986); Locht et al., Science, 232:1258-1264 (1986) and Pizza et al., Proc. Natl. Acad. Sci., 85:7521-7525 (1988). The present invention also contemplates a segment of the pertussis toxin S1 gene that encodes a polypeptide that is an enzyme that can catalyze the ADP-ribosylation and inactivation of G.sub.g and G.sub.i, the GTP-binding regulatory components that can stimulate protein kinase C and inhibit G.sub.s function. Other pertussis toxin genes or segments of genes that non-randomly hybridize to nucleotides 609 to 1313 of FIG. 2 or their complement are also contemplated by the present invention.

Detailed Description Text (18):
The present invention contemplates a cell containing a recombinant nucleic acid molecule of the present invention. The recombinant nucleic acid molecule contains a tissue specific promoter located 5' of a non-lethal modulator gene and a non-lethal modulator gene. The recombinant nucleic acid molecule can be operatively linked to a vector for amplification and/or expression. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors".

Detailed Description Text (19):
The choice of vector to which a non-lethal modulator gene of the present invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., replication or protein expression, and the host cell to be transformed, these being limitations inherit in the art of constructing recombinant DNA molecules. In preferred embodiments, the vector utilized includes a prokaryotic replicon i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a prokaryotic host cell, such as a bacterial host cell. Such replicons are well known in the art. In addition,

preferred embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as a drug resistance, to the bacterial host cell when introduced into those transformed cells. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Detailed Description Text (20):
Those vectors that include a prokaryotic replicon also typically include convenient restriction sites for insertion of a recombinant DNA molecule of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories, (Richmond, CA) and pPL, pK and K223 available from Pharmacia, (Piscataway, N.J.), and pBLUESCRIPT and pBS available from Stratagene,(La Jolla, Calif.). A vector of the present invention may also be a Lambda phage vector including those Lambda vectors described in Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis et al., eds., Cold Spring Harbor, N.Y. (1989), and the Lambda ZAP vectors available from Stratagene, (La Jolla, Calif.). Other exemplary vectors include pCMU (Nilsson, et al., Cell 58:707 (1989)). Other appropriate vectors may also be synthesized, according to known methods; for example, vectors pCMU/K.sup.b and pCMUII used in various applications herein are modifications of pCMUIV (Nilsson, et al., supra).

Detailed Description Text (21):
Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells or plant cells can be used. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors provide convenient restriction sites for insertion of the desired recombinant DNA molecule. Typical of such vectors are pSVO and pKSV-10 (Pharmacia), and pPVV-1/PML2d (International Biotechnology, Inc.), and pTDT1 (ATCC, No. 31255).

Detailed Description Text (22):
Typical expression vectors capable of expressing a recombinant nucleic acid sequence in plant cells include vectors derived from the tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens described by Rogers et al., Meth. in Enzymol., 153:253-277 (1987), and several other expression vector systems known to function in plants. See for example, Verma et al., Published PCT Application No. W087/00551; Cocking and Davey, Science, 236:1259-1262 (1987).

Detailed Description Text (23):
In preferred embodiments, the eukaryotic cell expression vectors used include a selection marker that is effective in a eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance selection marker is a gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (neo) gene. See for example, Southern et al., J. Mol. Appl. Genet., 1:327-341 (1982). (1982). In preferred embodiments where a recombinant nucleic acid molecule of the present invention is expressed in plant cells, a preferred drug resistance marker is the gene whose expression results kanamycin resistance, i.e., the chimeric gene containing nopaline synthetase promoter, Tn5 neomycin phosphotransferase II and nopaline synthetase 3' non-translated region described by Rogers et al., Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, Calif. (1988).

Detailed Description Text (24):
The use of retroviral expression vectors to express recombinant nucleic acid molecules of the present invention is also contemplated. As used herein, the term "retroviral expression vector" refers to a DNA molecule that includes a promoter sequence derived from the long terminal repeat "LTR" region of a retrovirus gene and which is encapsulated in the viral protein coat capable of introducing the DNA into cells and integrating it into the cell's genome. In preferred embodiments, the expression vector is typically a retroviral expression vector that is preferably replication-incompetent in eukaryotic cells. The

construction and use of retroviral vectors has been described by Sorge et al., Mol. Cell Biol., 4: 1730-1737 (1984).

Detailed Description Text (25):
In another preferred embodiment the expression vector is a retroviral expression vector that is replication incompetent and which already carries a marker gene such as the .beta.-galactosidase gene. This marker gene allows cells infected by the retroviral vector to be identified by detecting the presence of the marker gene. Typically the marker gene is placed in the retroviral vector so that the non-lethal modulator gene must be coexpressed with the marker gene.

Detailed Description Text (26):
Replication incompetent retroviral expression vectors can be used to infect eukaryotic cells or tissues such as neurons and cortical progenitor cells. The infection may occur in vivo or in vitro. Retroviral vectors carrying a .beta.-galactosidase marker gene have been used to infect neurons by Luskin et al., Neuron, 1:635-647 (1988).

Detailed Description Text (29):
Also contemplated by the present invention are tissues containing a recombinant nucleic acid molecule of the present invention. Tissues containing a recombinant nucleic acid molecule of the present invention may be prepared by introducing a recombinant nucleic acid molecule into a tissue, such as bone marrow, brain and liver, using known transformation techniques. These transformation techniques include transfection and infection by retroviruses carrying either a marker gene or a drug resistance gene. See for example, Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley and Sons, New York (1987) and Friedmann, T., Science, 244:1275-1281 (1989). A tissue containing a recombinant nucleic acid molecule of the present invention may then be reintroduced into an animal using reconstitution techniques. See for example, Dick et al., Cell, 42:71 (1985).

Detailed Description Text (30):
A tissue containing a recombinant nucleic acid molecule of the present invention may also be prepared by introducing a recombinant DNA molecule of the present invention into the germ line of a mammal. After introduction into the germ line the recombinant DNA molecule is present in all the tissues of that mammal. See for example, Palmiter, et al., Ann. Rev. Genet., 20:465-499 (1986).

Detailed Description Text (31):
Isolation of tissues from an animal whose tissues contain the recombinant nucleic acid molecule is accomplished using standard techniques. For example, the liver, lungs, spleen, or bone marrow can be removed using standard surgical techniques.

Detailed Description Text (32):
A tissue containing a recombinant DNA molecule of the present invention may also be produced by directly introducing a vector containing the recombinant DNA molecule into the animal. Direct vector delivery in vivo may be accomplished by transducing the desired cells and tissues with viral vectors or other physical gene transfer vehicles in vivo. Other physical agents including naked plasmids, cloned genes encapsulated in targetable liposomes or in erythrocyte ghosts have been use to introduce genes, proteins, toxins and other agents directly into whole animals. See, for example, the liposome-mediated gene delivery in vivo and expression of preproinsulin genes in recipient rats described by Nikolau, et al., Proc. Natl. Acad. Sci., USA, 80:1068 (1983) and Soriano, et al., Proc. Natl. Acad. Sci., USA, 80:7128 (1983). Direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. See for example, Kaneda, et al., Science, 243:375 (1989).

Detailed Description Text (34):
The present invention also contemplates a mammal containing a recombinant nucleic acid molecule of the present invention. Mammals containing recombinant nucleic acid molecules of the present invention may be prepared using the standard transgenic technology described in Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor, N.Y. (1987), Palmiter et al., Ann. Rev. Genet., 20:465-499 (1986). Production of transgenic animals is also possible using the homologous recombination transgenic systems described by Capecchi, Science, 244:288-292 (1989).

Detailed Description Text (35):
One technique for transgenically altering a mammal is to microinject a recombinant nucleic acid molecule into the male pronucleus of the fertilized mammalian egg to cause 1 or more copies of the recombinant nucleic acid molecule to be retained in the cells of the developing mammal. The recombinant nucleic acid molecule of interest is isolated in a linear form with most of the sequences used for replication in bacteria removed. Linearization and removal of excess vector sequences results in a greater efficiency in production of transgenic mammals. See for example, Brinster, et al., Proc. Natl. Acad. Sci., USA, 82:4438-4442 (1985). Usually up to 40 percent of the mammals developing from the injected eggs contain at least 1 copy of the recombinant nucleic acid molecule in their tissues. These transgenic mammals usually transmit the gene through the germ line to the next generation. The progeny of the transgenically manipulated embryos may be tested for the presence of the construct by Southern blot analysis of a segment of tissue. Typically, a small part of the tail is used for this purpose. The stable integration of the recombinant nucleic acid molecule into the genome of the transgenic embryos allows permanent transgenic mammal lines carrying the recombinant nucleic acid molecule to be established.

Detailed Description Text (36):
Alternative methods for producing a mammal containing a recombinant nucleic acid molecule of the present invention include infection of fertilized eggs, embryo-derived stem cells, totipotent embryonal carcinoma (Ec) cells, or early cleavage embryos with viral expression vectors containing the recombinant nucleic acid molecule. See for example, Palmiter et al., Ann. Rev. Genet., 20:465-499 (1986) and Capecchi, Science, 244:1288-1292 (1989).

Detailed Description Text (38):
The present invention also contemplates a method of genetically programming a cell within an organism by introducing a recombinant nucleic acid molecule of the present invention into the genome of a zygote to produce a genetically altered zygote or into the genome of individual somatic cells in the organism. The genetically altered zygote is then maintained under appropriate biological conditions for a time period equal to a gestation period or a substantial portion of a gestation period that is sufficient for the genetically altered zygote to develop into a transgenic organism containing at least 1 recombinant nucleic acid molecule of the present invention. The transgenic organism is then maintained for a time period sufficient for the non-lethal modulator gene present in the recombinant nucleic acid molecule to be expressed within a cell or cell type of the transgenic organism and thereby genetically program the cell within the organism.

Detailed Description Text (39):
The term "genetically programming" as used herein means to permanently alter the DNA, RNA, or protein content of a cell within an organism such as a mammal and thereby produce a biological effect. Typically, this genetic programming is accomplished by introducing a recombinant nucleic acid molecule of the present invention into the genome of the organism.

Detailed Description Text (40):
Any multicellular eukaryotic organism which undergoes sexual reproduction by the union of gamete cells may be genetically programmed using a nucleic acid molecule containing a non-lethal modulator

gene. Examples of such multicellular eukaryotic organisms include amphibians, reptiles, birds, mammals, bony fishes, cartilaginous fishes, cyclostomes, arthropods, insects, mollusks, thallophytes, embryophytes including gymnosperms and angiosperms. In preferred embodiments, the multicellular eukaryotic organism is a mammal, bird, fish, gymnosperm or an angiosperm.

Detailed Description Text (41):
A transgenic organism is an organism that has been transformed by the introduction of a recombinant nucleic acid molecule into its genome. Typically, the recombinant nucleic acid molecule will be present in all of the germ cells and somatic cells of the transgenic organism. Examples of transgenic organisms include transgenic mammals, transgenic fish, transgenic mice, transgenic rats and transgenic plants including monocots and dicots. See for example, Gasser et al., Science, 244:1293-1299 (1989); European Patent Application No. 0257472 filed Aug. 13, 1987 by De La Pena et al.; PCT Pub. No. WO 88/02405 filed Oct. 1, 1987 by Trulson et al.; PCT Pub. No. WO 87/00551 filed Jul. 16, 1986 by Verma, and PCT Pub. No. WO 88/09374 filed May 20, 1988 by Topfer et al.

Detailed Description Text (42):
Methods for producing transgenic organisms containing a recombinant nucleic acid molecule of the present invention include standard transgenic technology; infection of the zygote or organism by viruses including retroviruses; infection of a tissue with viruses and then reintroducing the tissue into an animal; and introduction of a recombinant nucleic acid molecule into an embryonic stem cell of a mammal followed by appropriate manipulation of the embryonic stem cell to produce a transgenic animal. See for example, Wagner, et al., U.S. Pat. No. 4,873,191 (Oct. 10, 1989); Rogers, et al., Meth. in Enzymol., 153:253-277 (1987); Verma et al., Published PCT Application No. WO87/00551; Cocking et al., Science, 236:1259-1262 (1987); and Luskin et al., Neuron 1:635-647 (1988), the disclosures of which are incorporated by reference herein.

Detailed Description Text (43):
A cell within the organism that contains the recombinant nucleic acid molecule of the present invention is maintained for a time period sufficient for the non-lethal modulator gene present in the recombinant nucleic acid molecule to be expressed within that cell and thereby genetically program the cell of the organism.

Detailed Description Text (44):
Typically, the cell is maintained under biological growth conditions, appropriate for that organism for a sufficient time period. The biological growth conditions must allow the cell containing the recombinant nucleic acid molecule to express the non-lethal modulator gene present on the recombinant nucleic acid molecule. This time period is typically of a length to allow the non-lethal gene present in the recombinant nucleic acid molecule to be expressed. During expression the non-lethal modulator gene is first transcribed into RNA by RNA polymerase and then the RNA is translated into protein to produce the non-lethal modulator. The non-lethal modulator then modulates a complex biochemical pathway of the cell in which the chain is expressed but is not lethal to that cells.

Detailed Description Text (45):
Preferred embodiments of the present invention contemplate a method of altering a complex biochemical pathway within a cell of a transgenic mammal by producing a transgenic mammal having at least 1 cell containing and expressing a recombinant nucleic acid molecule of the present invention. The recombinant nucleic acid molecule containing transgenic mammal is maintained for a time period sufficient for the non-lethal modulator gene present in the recombinant nucleic acid molecule to be expressed in the cell and thereby altering a complex biochemical pathway within the cell of the transgenic mammal.

Detailed Description Text (46):
Transgenic mammals having at least 1 cell containing a recombinant nucleic acid molecule of the present invention can be produced using methods well known in the art. See for example, Wagner et al., U.S. Pat. No. 4,873,191 (Oct. 10, 1989); Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Springs Harbor, N.Y. (1987); Capecchi, Science, 244:288-292 (1989); and Luskin et al., Neuron 1:635-647 (1988).

Detailed Description Text (48):
1) microinjecting a recombinant nucleic acid molecule into a fertilized mammalian egg to produce a genetically altered mammalian egg;

Detailed Description Text (51):
4) harvesting a transgenic mammal having at least one cell containing a recombinant nucleic acid molecule of claim 1 that has developed from the genetically altered mammalian egg.

Detailed Description Text (52):
A recombinant nucleic acid molecule of the present invention is provided, typically in linearized form, by linearizing the recombinant nucleic acid molecule with at least 1 restriction endonuclease. In addition, the recombinant nucleic acid molecule containing the tissue specific promoter and non-lethal modulator gene may be isolated from the vector sequences using 1 or more restriction endonucleases. Techniques for manipulating and linearizing recombinant nucleic acid molecules are well known and include the techniques described in Molecular Cloning: A Laboratory Manual, Second Edition, Maniatis et al., eds., Cold Spring Harbor, N.Y. (1989).

Detailed Description Text (54):
The linearized recombinant nucleic acid molecule may be microinjected into the mammalian egg to produce a genetically altered mammalian egg using well known techniques. Typically, the linearized nucleic acid molecule is microinjected directly into the pronuclei of the fertilized mouse eggs as has been described by Gordon et al., Proc. Natl. Acad. Sci., USA, 77:7380-7384 (1980). This leads to the stable chromosomal integration of the recombinant nucleic acid molecule in approximately 10 to 40 percent of the surviving embryos. See for example, Brinster et al., Proc. Natl. Acad. Sci., USA, 82:4438-4442 (1985). In most cases, the integration appears to occur at the 1 cell stage, as a result the recombinant nucleic acid molecule is present in every cell of the transgenic animal, including all of the primordial germ cells. The number of copies of the foreign recombinant nucleic acid molecule that are retained in each cell can range from 1 to several hundred and does not appear to depend on the number of recombinant nucleic acid molecules injected into the egg.

Detailed Description Text (55):
An alternative method for introducing genes into the mouse germ line is the infection of embryos with virus vectors. The embryos can infected by either wild-type or recombinant viruses leading to the stable of integration of viral genomes into the host chromosomes. See, for example, Jaenisch et al., Cell, 24:519-529 (1981). One particularly useful class of viral vectors are virus vector derived from retro-viruses. Retroviral integration occurs through a precise mechanism, leading to the insertion of single copies of the virus on the host chromosome. The frequency of obtaining transgenic animals by retroviral infection of embryos can be as high as that obtained by microinjection of the recombinant nucleic acid molecule and appears to depend greatly on the titre of virus used. See, for example, van der Putten et al., Proc. Natl. Acad. Sci., USA, 82:6148-6152 (1985).

Detailed Description Text (56):
Another method of transferring new genetic information into the mouse embryo involves the introduction of the recombinant nucleic acid molecule into embryonic stem cells and then introducing

the embryonic stem cells into the embryo. The embryonic stem cells can be derived from normal blastocysts and these cells have been shown to colonize the germ line regularly and the somatic tissues when introduced into the embryo. See, for example, Bradley et al., Nature, 309:255-256 (1984). Typically, the embryo-derived stem cells are transfected with the recombinant nucleic acid molecule and the embryo-derived stem cells further cultured for a time period sufficient to allow the recombinant nucleic acid molecule to integrate into the genome of the cell. In some situations this integration may occur by homologous recombination with a gene that is present in the genome of the embryo-derived stem cell. See, for example, Capecchi, Science, 244:1288-1292 (1989). The embryo stem cells that have incorporated the recombinant nucleic acid molecule into their genome may be selected and used to produce a purified genetically altered embryo derived stem cell population. See, for example, Mansour et al., Nature, 336:348 (1988). The embryo derived stem cell is then injected into the blastocoel cavity of a preimplantation mouse embryo and the blastocyst is surgically transferred to the uterus of a foster mother where development is allowed to progress to term. The resulting animal is chimeric in that it is composed from cells derived of both the donor embryo derived stem cells and the host blastocyst. Heterozygous siblings are interbred to produce animals that are homozygous for the recombinant nucleic acid molecule. See for example, Capecchi, Science, 244:1288- 1292 (1989).

Detailed Description Text (58):
The host female mammals containing the implanted genetically altered mammalian eggs are maintained for a sufficient time period to give birth to a transgenic mammal having at least 1 cell containing a recombinant nucleic acid molecule of the present invention that has developed from the genetically altered mammalian egg. Typically this gestation period is between 19 to 20 days depending on the particular mouse strain. The breeding and care of mice is well known. See for example, Manipulating the Mouse Embryo: A Laboratory Manual, Hogan et al., eds., Cold Spring Harbor, N.Y., (1986).

Detailed Description Text (59):
In other preferred embodiments, the transgenic animal of the present invention is produced by infecting an animal vector containing a marker gene and a tissue specific promoter operatively linked to a non-lethal modulator gene to produce a genetically altered animal cell.

Detailed Description Text (62):
The infection of cells within an animal using a replication incompetent retroviral vector has been described by Luskin et al., Neuron, 1:635-647 (1988).

Detailed Description Text (64):
Typically the genetically altered animal is maintained for at least 24 hours to allow the retrovirus vector to incorporate into the genome of the cells that it infects.

Detailed Description Text (165):
Typically, the compound is introduced by any suitable means including ingestion, injection or trans-dermal administration.

Detailed Description Text (167):
This invention also contemplates a method of determining the therapeutic effectiveness of a composition by: (1) treating an animal comprised of cells containing a vector having a tissue specific promoter operatively linked to a non-lethal modulator gene with a predetermined, physiologically effective amount of a composition. (2) the treated animal is then maintained for a predetermined period of time sufficient to allow the composition to produce a physiologic effect when the modulator gene is being expressed. (3) Any change in the physiologic condition of the maintained animal is determined thereby determining the therapeutic effectiveness of the composition.

Detailed Description Text (168):
An animal comprised of cells containing a nucleic acid molecule having a tissue specific promoter operatively linked to a non-lethal modulator gene is treated with a predetermined, physiologically effective amount of a composition. Such animals are prepared according to the method of this invention. See for example, Examples 1-4.

Detailed Description Text (169):
The animals may be treated with the composition by injection, ingestion or trans-dermal administration. Preferably, the composition is either provided in the animal's diet or injected.

Detailed Description Text (174):
F. Vectors

Detailed Description Text (175):
In various embodiments, the present invention contemplates the use of a variety of vectors, e.g., as helpers or as means of delivering nucleotide sequences according to the present invention. In various preferred embodiments, vectors may be plasmids, episomes, phage, virus, retrovirus, adenovirus, or may comprise one or more portions of same--e.g., a useful vector may be a derivative or a portion of a virus, such as the penton complex of adenovirus. Vectors according to the present invention may further be described to include cloning vectors and expression vectors.

Detailed Description Text (176):
For example, as discussed in Sections A and B above, a subject nucleic acid molecule of the present invention typically comprises at least two different operatively linked DNA segments. The DNA can be manipulated and amplified using the standard techniques described in Molecular Cloning: A Laboratory Manual, 2nd Edition, Maniatis et al., eds., Cold Spring Harbor, N.Y. (1989). Typically, to produce a nucleic acid molecule of the present invention, the tissue specific promoter and the non-lethal modulator gene are operatively linked to a vector DNA molecule capable of autonomous replication in a cell. By operatively linking the tissue specific promoter and the non-lethal modulator gene to the vector the attached segments are replicated along with the vector sequences. Thus, a recombinant DNA molecule (rDNA) is a hybrid DNA molecule comprising at least two nucleotide sequences not normally found together in nature.

Detailed Description Text (177):
In preferred embodiments, the recombinant nucleic acid molecule that is introduced into the cells of a mammal contains a tissue specific promoter and a non-lethal modulator gene and has had the excess vector sequences removed. These excess vector sequences are removed by cutting the recombinant nucleic acid molecule with one or more restriction endonucleases to produce a linear recombinant nucleic acid molecule containing a tissue specific promoter and a non-lethal modulator gene.

Detailed Description Text (178):
The within-described recombinant nucleic acid molecules can be operatively linked to a vector for amplification and/or expression. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of autonomous replication and/or expression of nucleic acids or structural gene products present in the nucleotide segments to which they are operatively linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". As used herein with regard to DNA sequences or segments, the phrase "operatively linked" means the sequences or segments have been covalently joined into one piece of DNA, whether in single or double stranded form.

Detailed Description Text (179):
The choice of vector to which a non-lethal modulator gene of the present invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., replication or protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules. In preferred embodiments, the vector utilized includes a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extra-chromosomally when introduced into a prokaryotic host cell, such as a bacterial host cell. Such replicons are well known in the art. In addition, preferred embodiments that include a prokaryotic replicon also include a gene whose expression confers a selective advantage, such as a drug resistance, to the bacterial host cell when introduced into those transformed cells. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline. Vectors also typically contain convenient restriction sites for insertion of translatable nucleotide sequences.

Detailed Description Text (180):
Those vectors that include a prokaryotic replicon also typically include convenient restriction sites for insertion of a recombinant DNA molecule of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories (Richmond, CA); pPL, pK and pKK223 available from Pharmacia (Piscataway, N.J.); and pBLUESCRIPT, M13mp19 and pBS available from Stratagene (La Jolla, Calif.). A vector of the present invention may also be a Lambda phage vector including those Lambda vectors described in Molecular Cloning: A Laboratory Manual, 2d Ed., Maniatis, et al., eds., Cold Spring Harbor, N.Y. (1989), and the Lambda ZAP vectors available from Stratagene (La Jolla, Calif.). Other useful vectors are described in U.S. Pat. No. 4,338,397, the disclosure of which is incorporated herein by reference.

Detailed Description Text (181):
Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells or plant cells, may be used according to the present invention. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors provide convenient restriction sites for insertion of the desired recombinant DNA molecule. Typical of such vectors are pSVO and pKSV-10 (Pharmacia), pPVV-1/PML2d (International Biotechnology, Inc.), and pTDT1 (ATCC Accession No. 31255).

Detailed Description Text (182):
Mammalian expression vector systems are also contemplated for the expression of nucleotide sequences according to the present invention. For controlling expression in mammalian cells, viral-derived promoters are most commonly used. For example, frequently used promoters include polyoma, adenovirus type 2, and Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication. Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 base pair sequence extending from the Hind III restriction site toward the Bgl I site located in the viral origin of replication. Also contemplated is using the promoter sequences associated with the desired sequence for expression, in this instance, tissue-specific promoters. Origins of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral sources such as polyoma and adenovirus, or may be provided by the host cell chromosomal replication mechanism. The latter is sufficient for integration of the expression vector in the host cell chromosome.

Detailed Description Text (183):
Typical expression vectors capable of expressing a recombinant nucleic acid sequence in plant cells

include vectors derived from the tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens described by Rogers et al., Meth. in Enzymol., 153:253-277 (1987), and several other expression vector systems known to function in plants. See for example, Verma et al., Published PCT Application No. WO87/00551; Cocking and Davey, Science, 236:1259-1262 (1987).

Detailed Description Text (184):
In preferred embodiments, the eukaryotic cell expression vectors used include a selection marker that is effective in a eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance selection marker is a gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (neo) gene. See for example, Southern et al., J. Mol. Appl. Genet., 1:327-341 (1982). (1982). In preferred embodiments where a recombinant nucleic acid molecule of the present invention is expressed in plant cells, a preferred drug resistance marker is the gene whose expression results in kanamycin resistance, i.e., the chimeric gene containing nopaline synthetase promoter, Tn5 neomycin phosphotransferase II and nopaline synthetase 3' non-translated region described by Rogers et al., Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, Calif. (1988).

Detailed Description Text (185):
The use of retroviral and adenoviral expression vectors to express recombinant nucleic acid molecules of the present invention is also contemplated. As used herein, the terms "retroviral expression vector" and "adenoviral expression vector" respectively, refer to a DNA molecule that includes a promoter sequence derived from either the long terminal repeat (LTR) region of a retrovirus nucleotide sequence or from the promoter region of an adenovirus nucleotide sequence, and which is encapsulated in a viral protein coat capable of introducing the DNA into cells and either integrating it into the cell's genome (in the case of retroviral expression vectors) or maintaining the introduced DNA in an episome, or non-integrated plasmid (in the case of adenoviral expression vectors). In preferred embodiments, the expression vector is a retroviral or adenoviral expression vector that is preferably replication-incompetent in eukaryotic cells. The construction and use of retroviral vectors has been described by Sorge et al., Mol. Cell Biol., 4:1730-1737 (1984); the construction and use of adenoviral vectors has been described by Davidson, et al., Nature Genetics 3:219-222 (1993).

Detailed Description Text (186):
In another embodiment, the expression vector is a retroviral or adenoviral expression vector that is replication incompetent and which already carries a marker gene such as the .beta.-galactosidase gene. This marker gene allows cells infected by the retroviral or adenoviral vector to be identified by detecting the presence of the marker gene. Typically, the marker gene is placed in the retroviral or adenoviral vector so that the non-lethal modulator gene must be coexpressed with the marker gene.

Detailed Description Text (187):
Replication incompetent retroviral or adenoviral expression vectors can be used to infect eukaryotic cells or tissues including, for example, neurons and cortical progenitor cells. The infection may occur in vivo or in vitro. Retroviral vectors carrying a .beta.-galactosidase marker gene have been used to infect neurons by Luskin et al., Neuron, 1:635-647 (1988). Adenoviral vectors have been used similarly; see, e.g., Davidson, et al., Nature Genetics 3:219-222 (1993).

Detailed Description Text (188):
Viral vectors and virally-derived vectors are also known in the art and are useful according to the present invention.

Detailed Description Text (189):
Nucleic acid production using plasmid or phage vectors has become very straightforward. The plasmid

or phage DNA is cleaved with a restriction endonuclease and joined in vivo to a foreign DNA of choice. The resulting recombinant plasmid or phage is then introduced into a cell such as E. coli, and the cell so produced is induced to produce many copies of the engineered vector. Once a sufficient quantity of DNA is produced by the cloning vector, the produced foreign DNA is excised and placed into a second vector to produce or transcribe the protein or polypeptide encoded by the foreign gene.

Detailed Description Text (190):
Depending on the DNA (intact gene, cDNA, or bacterial gene), it may be necessary to provide eucaryotic transcription and translation signals to direct expression in recipient cells either in vivo or in vitro. These signals may be provided by combining the foreign DNA in vitro with an expression vector.

Detailed Description Text (191):
Expression vectors contain sequences of DNA that are required for the transcription of cloned genes and the translation of their messenger RNAs (mRNAs) into proteins. Typically, such required sequences or control elements are: (1) a promoter that signals the starting point for transcription; (2) a terminator that signals the ending point of transcription; (3) an operator that regulates the promotor; (4) a ribosome binding site for the initial binding of the cells' protein synthesis machinery; and (5) start and stop codons that signal the beginning and ending of protein synthesis.

Detailed Description Text (192):
To be useful, an expression vector should possess several additional properties. It should be relatively small and contain a strong promoter. The expression vector should carry one or more selectable markers to allow identification of transformants. It should also contain a recognition site for one or more restriction enzymes in regions of the vector that are not essential for expression.

Detailed Description Text (193):
The construction of expression vectors is, therefore, a complicated and somewhat unpredictable venture. The only true test of the effectiveness of an expression vector is to measure the frequency with which the synthesis of the appropriate mRNA is initiated. However, quantitation of mRNA is tedious, and it is often difficult to obtain accurate measurements. Other more practicable means have, therefore, been developed to detect transformation.

Detailed Description Text (195):
Another means used to monitor transformation involves the use of immunological reagents. If the level of expressed protein is sufficiently high, then cytoplasmic or surface immunofluorescence with an antibody conjugated to a fluorescent dye such as fluorescein or rhodamine may be used to detect vector-specific protein expression products.

Detailed Description Text (197):
A vector of the present invention is a nucleic acid (preferably DNA) molecule capable of autonomous replication in a cell and to which a DNA segment, e.g., gene or polynucleotide, can be operatively linked so as to bring about replication of the attached segment. In the present invention, one of the nucleotide segments to be operatively linked to vector sequences encodes at least a portion of a nucleic acid molecule of the present invention. Preferably, the entire peptide-coding sequence of the subject nucleic acid molecule is inserted into the vector and expressed; however, it is also feasible to construct a vector which also includes some non-coding sequences as well. Preferably, the non-coding sequences are excluded. Alternatively, a nucleotide sequence for a soluble form of a polypeptide may be utilized. Another preferred vector includes a nucleotide sequence encoding at least a portion of a nucleic acid molecule of the present invention operatively linked to the vector for expression.

Detailed Description Text (198):

A sequence of nucleotides adapted for directional ligation, i.e., a polylinker, is a region of the expression vector that (1) operatively links for replication and transport the upstream and downstream nucleotide sequences and (2) provides a site or means for directional ligation of a nucleotide sequence into the vector. Typically, a directional polylinker is a sequence of nucleotides that defines two or more restriction endonuclease recognition sequences, or restriction sites. Upon restriction cleavage, the two sites yield cohesive termini to which a translatable nucleotide sequence can be ligated to the expression vector. Preferably, the two restriction sites provide, upon restriction cleavage, cohesive termini that are non-complementary and thereby permit directional insertion of a translatable nucleotide sequence into the vector. In one embodiment, the directional ligation means is provided by nucleotides present in the upstream nucleotide sequence, downstream nucleotide sequence, or both. In another embodiment, the sequence of nucleotides adapted for directional ligation comprises a sequence of nucleotides that defines multiple directional cloning means. Where the sequence of nucleotides adapted for directional ligation defines numerous restriction sites, it is referred to as a multiple cloning site.

Detailed Description Text (199):
A translatable nucleotide sequence is a linear series of nucleotides that provide an uninterrupted series of at least 8 codons that encode a polypeptide in one reading frame. Preferably, the nucleotide sequence is a DNA sequence. The vector itself may be of any suitable type, such as a viral vector (RNA or DNA), naked straight-chain or circular DNA, or a vesicle or envelope containing the nucleic acid material and any polypeptides that are to be inserted into the cell.

Detailed Description Text (201):
Most useful vectors contain multiple elements including one or more of the following, depending on the nature of the recipient cell: an SV40 origin of replication for amplification to high copy number; an efficient promoter element for high-level transcription initiation; mRNA processing signals such as mRNA cleavage and polyadenylation sequences (and frequently, intervening sequences as well); polylinkers containing multiple restriction endonuclease sites for insertion of foreign DNA; selectable markers that can be used to select cells that have stably integrated the plasmid DNA; and plasmid replication control sequences to permit propagation in bacterial cells. In addition to the above, many vectors also contain an inducible expression system that is regulated by an external stimulus. Sequences from a number of promoters that are required for induced transcription have been identified and engineered into expression vectors to obtain inducible expression. Several useful inducible vectors have been based on induction by .gamma.-interferon, heat-shock, heavy metal ions, and steroids (e.g. glucocorticoids). (See, e.g., Kaufman, Meth. Enzymol. 185:487-511 (1990).) Other promoters contemplated for use in this invention are described in Example 5.

Detailed Description Text (202):
A preferred vector in which therapeutic nucleotide compositions of this invention are present is a plasmid; more preferably, it is a high-copy-number plasmid. It is also desirable that the vector contain an inducible promoter sequence, as inducible promoters tend to limit selection pressure against cells into which such vectors (which are often constructed to carry non-native or chimeric nucleotide sequences) have been introduced. It is also preferable that the vector of choice be best suited for expression in the preselected recipient cell type depending on the nature of the gene replacement therapy.

Detailed Description Text (203):
A tissue containing a therapeutic nucleotide sequence of the present invention may also be produced by directly introducing the vector containing the sequence into an animal or into isolated donor human, fetal, or animal tissues. Direct vector delivery in vivo may be accomplished by transducing the desired cells and tissues with viral vectors or other physical gene transfer vehicles. Other physical agents including naked plasmids, cloned genes encapsulated in targetable liposomes (see Section E below) or in erythrocyte ghosts have been use to introduce genes, proteins, toxins and other agents directly into

whole animals.

Detailed Description Text (207):
Construction of the Non-Lethal Modulator Gene Expression Vector

Detailed Description Text (208):
A 600 base pair nucleic acid segment encoding the mature and enzymatically active A1 subunit of cholera toxin, a non-lethal modulator gene, was placed immediately 3' of the tissue specific promoter of the rat growth hormone gene to produce a tailored fusion gene encoding cholera toxin. To produce this gene, the 600 base pair region encoding the mature and enzymatically active A1 subunit of cholera toxin was amplified from the cloned ctx operon Vibrio cholerae holotoxin using the polymerase chain reaction. The cloned sequence for the Vibrio cholera holotoxin has been completely sequenced and previously described by Mekalanos et al., Nature, 306:551-557 (1983) and is present in clone PRT41. The A1 subunit of cholera toxin is encoded by nucleotides 570 to 1151 of FIG. 1.

Detailed Description Text (209):
Polymerase chain reaction (PCR) primers were designed to remove sequences encoding the signal peptide for bacterial secretion, and the A2 subunit which is normally cleaved off after cell penetration, and the B subunit responsible for cell penetration. The PCR primers in Table II were synthesized using an automated oligonucleotide synthesizer. The PCR primers amplified nucleotides 570 to 1151 and added a consensus eukaryotic translation initiation site (CCACCATGG) and termination sites as described by Kozak, M, Nucleic Acids Research, 12:857-852 (1984). The PCR primers also added a termination codon (TAG) to the amplified A1 coding region as well as flanking Bam HI restriction endonuclease sites (GGATCC).

Detailed Description Text (210):
An exemplary PCR buffer comprises the following: 50 mM KCl; 10 mM Tris-HCl at pH 8.3; 1.5 mM MgCl.sub.2 ; 0,001% (wt/vol) gelatin, 200 .mu.M dATP; 200 .mu.M dTTP; 200 .mu.M dCTP; 200 .mu.M dGTP; and 2.5 units Thermus aquaticus DNA polymerase I (U.S. Pat. No. 4,889,818) per 100 microliters of buffer.

Detailed Description Text (211):
The PCR amplification was carried out by admixing in a 50 microliter reaction 100 nanograms (ng) of the cloned ctx operon of the Vibrio cholera holotoxin DNA (EcoRI-linearized PRT41 template DNA) to a solution containing 50mM KCl, 10mM Tris-HCl at pH 8.4, 1.5 mM MgCl.sub.2, 250 pmole of primers CT-5' and CT-3' shown in Table II, 200 .mu.M each of dATP, dCTP, dTTP, dGTP, 200 .mu.g/ml of gelatin and 2 units of Taq polymerase (Cetus Corp., Emeryville, Calif.). The samples were overlaid with approximately 50.mu.l of mineral oil to prevent evaporation and subjected to 30 cycles of amplification according to the manufacturers instructions. In each amplification cycle the solution was heated to 92.degree. C. over a 30 second time period to denature the DNA, cooled to 50.degree. C. over two minutes to allow the primers to anneal with the Vibrio cholera DNA, and maintained at 72.degree. C. for 2 minutes to produce a complementary DNA (cDNA) strand from the Vibrio cholera DNA. The resulting amplified DNA segment containing the A1 subunit of cholera toxin is flanked by Bam HI restriction endonuclease sites that allow the fragment to be easily inserted into a vector containing the tissue specific promoter.

Detailed Description Text (212):
The PCR reaction was tested for appropriate amplification by ethidium bromide staining and examination of 1/10th of the reaction, run on a 1% agarose electrophoresis gel. A band of the expected size (.about.600 bp) was seen, confirming appropriate amplification. The remainder of the PCR product was phenol-chloroform-isoamyl alcohol (64:32:4) extracted twice and ethanol precipitated to remove

residual Taq polymerase activity and free nucleotides. 2 .mu.g of the PCR product was then 5-fold over-digested with BamHI, the BamHI was inactivated by heating at 68.degree. C. and then freezing, and then 20 ng of the BamHI-cut PCR product was ligated to 20 ng of the BamHI-cut and phosphatased rGH-hGH vector DNA.

Detailed Description Text (213):
The amplified DNA segment containing the A1 subunit of cholera toxin flanked by Bam HI restriction endonuclease sites was inserted into the unique Bam HI restriction endonuclease site within the 5' untranslated region of the rGH-hGH fusion gene previously described by Behringer et al., Genes and Development, 2:453-461 (1988). It consists of a 310 bp KpnI-XhoI fragment containing the rat growth hormone (rGH) promoter and transcription start site previously described by Braun et al., Proc. Natl. Acad. Sci., USA, 78:4867-4871 (1981), fused to a 2150 bp XhoI-EcoRI fragment containing the 5' untranslated region (containing a unique BamHI site) and remaining gene and 3' intergenic sequences for human growth hormone (hGH) previously described by Seeburg, DNA, 1:239-249 (1982), cloned into a pUC plasmid vector. It was prepared for insertion of the BamHI CT cassette by 6-fold over-digestion with BamHI, plus 0.0125 units/.mu.g/hr calf intestinal alkaline phosphatase to prevent circularization during the ligation step. The Bam HI digested rGH-hGH plasmid was purified by extraction with phenol followed by ethanol precipitation. Twenty nanograms (ng) of the PCR amplified DNA segment was then ligated directly to 20 ng of Bam HI digested rGH-hGH plasmid using the ligation procedures described in Molecular Cloning: A Laboratory Manual, Maniatis et al., eds., Cold Spring Harbor, N.Y. (1982). Briefly, ligation was performed overnight at 15.degree. C., in a 10 .mu.l volume containing 1 weiss unit T4 DNA ligase (BRL), 50 mM Tris 7.5, 10 mM MgCl.sub.2, 10 mM DTT, and 1 mM rATP.

Detailed Description Text (214):
A fifth (2 .mu.l) of the ligation was diluted to 20 .mu.l with TE buffer, and 100 .mu.l of CaCl.sub.2 - treated transformation-competent cells (MC1061 strain) were added. After a 30 minute incubation on ice to allow ligated plasmid attachment to cells, the cells were incubated at 42.degree. C. for 90 seconds to allow DNA entry into the cells, then 1 ml of 37.degree. C. 2.times.LB broth was added and the cells placed at 37.degree. C. for 40 minutes. 300 .mu.l of the cells were then spread on LB-ampicillin agar plates. Ampicillin resistant colonies appeared on the plates after 12 hrs of 37.degree. C. incubation. These colonies were screened for the presence of the putative recombinant plasmid by first hybridizing a colony lift of the plate with radiolabeled probe prepared from nick-translation of the PCR product to identify clones that carried the PCR-cassette insert, and then by restriction digesting plasmid miniprep DNA prepared from positive clones. First the DNA samples were cut with BamHI and analyzed by 1% agarose gel electrophoresis to confirm they contained the .about.600 bp BamHI PCR-cassette. Then the DNA samples were double-digested with KpnI and XbaI, which generate restriction fragments of different size depending upon the orientation of the inserted BamHI PCR- cassette. By this means several clones of the recombinant plasmid with a correctly oriented insert were identified. The PCR CT cassettes were sequenced by the dideoxy method using synthetic primers designed to sequence into the BamHI cloning site (5' -GAAAGGCAGGAGCCTTGGGG-3' and 5' -TGTCCACAGGACCCTGAGTG-3'), and were confirmed to have been PCR amplified and cloned without errors (see, e.g., Sanger, et al., PNAS USA, 74:5463-5467 (1977)). One of these identical clones was chosen for embryo microinjection and designated rGH-CT.

Detailed Description Text (215):
A large scale plasmid preparation of the rGH-CT clone was prepared and the DNA isolated and purified by density gradient centrifugation in cesium chloride. The 3 kb rGH-CT fusion gene fragment was separated from pUC vector sequences by KpnI-EcoRI digestion and 1% agarose gel electrophoresis. The KpnI-EcoRI gene fragment was cut out of the gel and purified from the agarose by the NaI-glass powder purification method described by Vogelstein et al., Proc. Natl. Acad. Sci., USA, 76:615-619 (1979). The

gene fragment was diluted to 1 ng/.mu.l concentration centrifuged at 10,000 Xg to remove residual sediment, and the resulting supernatant used for mouse embryo microinjections. The resulting cholera toxin expression vector contains a tissue specific rat growth hormone promoter located immediately 5' of the cholera toxin nucleic acid segment as shown in FIG. 3. The cholera toxin nucleic acid segment is located 5' of and controls the expression of the exons, introns, and polyadenylation site of the gene for human growth hormone. The tissue specific rat growth hormone promoter has been previously used in transgenic mice to specifically express human growth hormone and diphtheria toxin in pituitary somatotropes by Behringer et al., Genes and Development, 2:453-461 (1988) and Palmiter et al., Cell, 50:435-443 (1987). The cholera toxin expression vector retains in its 3' untranslated region that contains the nucleic acid segment of the human growth hormone gene for correct messenger RNA (mRNA) splicing and 3' end cleavage and polyadenylation. The accuracy of the above construction steps was confirmed by DNA sequencing using the Sanger dideoxy method described by Sanger et al., Proc. Natl. Sci. USA, 74:5463-5467 (1977).

Detailed Description Text (219):
To determine which of the 68 mice contained transgenic DNA, genomic DNA was obtained from tail tissue removed from each mouse at 4 weeks of age: 1.5 to 2 cm of tail was clipped off, frozen at -70.degree. C., and chopped into 6-8 sections which were added to 700 .mu.l of 50 mM Tris 8.0, 10 mM EDTA, 100 mM NaCl, 1% SDS, and 20 .mu.l of 20 mg/ml Proteinase K in an eppendorf tube. The SDS-Proteinase K treatment continued overnight to solubilize proteins, and then the samples were extracted once each with phenol 8.0, phenol chloroform-isoamyl alcohol 8.0 (64:32:4) and chloroform-isoamyl alcohol (24:1). Then the genomic DNA in the samples was ethanol precipitated by addition of 2 volumes of cold absolute ethanol followed by inverting the tube several times, and the DNA was removed by suction using a pipetteman tip, followed by dipping in 80% ethanol to rinse, and then air drying for 30 minutes or longer. The genomic DNA was then resuspended in 200 .mu.l of 10 mM Tris 8.0, 0.1 mM EDTA, and 10 mM NaCl by gentle rocking on a nutator at 4.degree. C. overnight. This procedure yielded 70-100 .mu.g of genomic DNA per 1.5-2 cm tail piece. Equivalent amounts of genomic DNA from each animal were then loaded onto a nitrocellulose slot blot apparatus and the blotted DNA samples were hybridized with a radiolabeled probe prepared by nick translation of the PCR amplified CT DNA. All subsequent offspring of the positive transgenic founders among the original 68 mice were also analyzed by this method. This hybridization revealed that 13 of transgenic mice carried the transgene.

Detailed Description Text (220):
The number of transgenes present in each of the transgenic mice was determined by Southern blotting using the procedures first described by Southern, J. Molec. Biol., 98:503-515 (1975). Briefly, the isolated nucleic acid was digested with EcoRI or XbaI restriction endonuclease then fractionated by size in an agarose gel. The size separated DNA was then transferred to a sheet of nitrocellulose and hybridized with a radiolabeled DNA probe prepared from the cholera toxin gene. Five transgenic mice had an apparent transgene copy number of less than or equal to one based on this Southern blot hybridization while nine had an apparent transgene copy number of greater than or equal to two. Of the original 13 transgenic mice, 9 had markedly higher serum growth hormone levels than nontransgenic littermates and 8 of these 9 had greater body weights. (Table III).

Detailed Description Text (221):
Seven of the nine transgenic mice with an apparent transgene copy number of greater than 2 and one transgenic mouse with an apparent copy number of less than or equal to 1 were bred to non-transgenic mates. Ninety seven F.sub.1 generation mice were obtained and of those 97, 47 were transgenic. Southern blotting of DNA from these transgenic lines identified 10 transgenic loci of which 8 showed approximately 50 percent inheritance (Table III).

Detailed Description Text (240):
The neuron-specific enolase-cholera toxin (NSE-CT) transgene, designed to express cholera toxin (CT) in mature neurons of transgenic animals or, when carried on an adenovirus gene therapy vector, in mature neurons of humans, tissues, or experimental animals, is shown in FIG. 5A. The cholera toxin (CT) open reading frame (ORF) corresponds to the A1 region of the cholera holotoxin operon, ctx. A1 is the intracellular ADP-ribosyltransferase subunit which elevates cAMP levels by constitutive activation of the Gs protein. The PCR-amplified ORF, joined to appropriate eukaryotic expression sequences, was inserted into the 5' untranslated region of a previously tested transgene, NSE-LacZ, converting the CT ORF into the new 5' ORF of the gene, while converting the LacZ sequences into 3' untranslated sequences. The SV40 sequences provide the poly-A addition site. Both the NSE-LacZ and NSE-CT fusion genes are intronless, but nevertheless appear to function well in transgenic mice.

Detailed Description Text (242):
A neuroanatomical examination of the most severely affected NSE-CT transgenic mice, which die at age P1 to P3, was undertaken in order to establish whether this lethal neurological disorder was associated with any obvious neuroanatomical abnormalities induced by transgene expression in neurons. Prior analysis of six previous litters of one NSE-CT mouse founder had confirmed that all transgenic pups of this founder die at P1 to P3. A seventh litter of 10 pups was therefore sacrificed early during P1, prior to any apparent illnesses among the pups. Brains of the pups, which all appeared normal by gross anatomical examination, were fixed in freshly prepared 4% paraformaldehyde and cryostat sectioned. Comparable mid-sagittal sections of the brains were then Nissl-stained for neuroarchitectural visualization. Tail DNA of the pups was also isolated and analyzed for the presence of the CT transgene in order to identify which pups were transgenic. In order to predict which neurons should be NSE-positive during this developmental period, comparable mid-sagittal sections from embryonic day 18 (E18) and P2 pups of the NSE-LacZ transgenic mouse strain, which expresses .beta.-galactosidase from the NSE promoter, were also examined.

Detailed Description Text (244):
As a therapeutic gene sequence, the NSE-CT transgene, when used with adenoviral vectors, may alleviate Parkinson's Disease when injected into the human striatum, by partly compensating for the loss of pre-synaptic dopamine.

Detailed Description Text (246):
The RV-LacZ-CT retroviral transgene, designed to express CT plus a blue color tracer in any exposed target cells in vitro or in animal or human subjects, is shown in FIG. 5B. A Moloney Murine-Leukemia Virus (Mo-MuLV) vector, which retains the viral long terminal repeats (LTRs) but from which the gag-pol-env viral sequences have been removed, was used to express both .beta.-galactosidase and cholera toxin from the LacZ and CT open reading frames (ORF), respectively. Both genes are expressed under the control of the SV40 virus early promoter, and are co-translated using a picornavirus-derived internal ribosome entry site (IRES) .

Detailed Description Text (247):
RV-LacZ-CT virus was prepared by co-transfection of the RV-LacZ-CT plasmid with helper phage and exposed to Chinese Hamster Ovary (CHO) cells in culture. The infected cells turned blue from expression of .beta.-galactosidase, and the blue-labeled cells were examined for morphological alterations induced by cholera toxin-mediated elevation of cAMP levels. They were shown to acquire a stellate, differentiated morphology in response to RV-LacZ-CT exposure but not to exposure to RV-LacZ, a control virus strain that does not express CT. This morphological alteration of the CHO cells can be duplicated by chemical cAMP-elevating agents such as exogenously added holotoxin or forskolin, indicating that the RV-LacZ-CT virus was inducing CT-mediated changes in CHO cell cAMP levels and subsequent cell differentiation and cellular activity.

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L20: Entry 5 of 32 File: USPT Jul 8, 2003

US-PAT-NO: 6589529
DOCUMENT-IDENTIFIER: US 6589529 B1

TITLE: Rotavirus subunit vaccine

DATE-ISSUED: July 8, 2003

INVENTOR-INFORMATION:

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L20: Entry 5 of 32 File: USPT Jul 8, 2003

DOCUMENT-IDENTIFIER: US 6589529 B1
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Brief Summary Text (5):
Rotaviruses are double-stranded RNA viruses and contain a multi-segmented genome. Because the viral genome is arranged in segments, these viruses are capable of genetic reassortment. This reassortment occurs when two or more rotaviruses infect a single cell and the various viral segments reassort during the packaging of new virus particles assembled in the cytoplasm of infected cells. The ability of the virus to reassort its genome components results in a great diversity of immune responses generated against the virus. This ability has also made generation of a rotavirus vaccine extremely challenging.

Brief Summary Text (6):
A variety of different approaches have been taken to generate a rotavirus vaccine suitable to protect human populations from the various serotypes of rotavirus. These approaches include various Jennerian approaches, use of live attenuated viruses, use of virus-like particles, nucleic acid vaccines and viral sub-units as immunogens.

Brief Summary Text (10):
U.S. Pat. Nos. 4,624,850, 4,636,385, 4,704,275, 4,751,080, 4,927,628, 5,474,773, and 5,695,767, each describe a variety of rotavirus vaccines and/or methods of preparing the same. A commonality shared by the members of this group is that each of these vaccines relies on the use of whole viral particles to create the ultimate rotavirus vaccines. Given the long standing need for an effective, multivalent vaccine, it is clear that this body of work has been only partially successful in addressing the need for such a vaccine.

Brief Summary Text (11):
Departing from traditional methods of vaccine generation, advances in the field of molecular biology have permitted the expression of individual rotavirus proteins. Using these techniques, vaccine candidates generated from virus-like particles of different protein compositions have shown potential as subunit vaccines. In one reference, VLPs containing VPs 2 and 6 or VPs 2, 6, and 7 were administered to mice with and without the addition of cholera toxin. O'Neal et al., "Rotavirus Virus-like like Particles Administered Mucosally Induce Protective Immunity," J. Virology, 71 (11):8707-8717 (1997). Both types of VLPs induced protective immunity in immunized mice, although protection was more effective when the VLPs were administered with cholera toxin (CT). In a subsequent study by the same group, the Escherichia coli heat-labile toxin (LT) was compared to CT for effectiveness in producing rotavirus protection. O'Neal et al., "Rotavirus 2/6 Virus-like Particles Administered Intranasally with Cholera Toxin, Escherichia coli Heat-Labile Toxin (LT), and LT-R192G Induce Protection from Rotavirus Challenge," J. Virology, 72(4):3390-3393 (1998). This group concluded that both the wild-type LT and a recombinant form of the molecule were effective adjuvants when immunizing with rotavirus VLPs.

Brief Summary Text (12):
Core-like particles and VLPs have also been used to immunize cows. Fernandez, et

al., "Passive Immunity to Bovine Rotavirus in Newborn Calves Fed Colostrum Supplements From Cows Immunized with Recombinant SA11 rotavirus core-like particle (CLP) or virus-like particle (VLP) vaccines," Vaccine, 16(5):507-516 (1998). In this this study the ability of CLPs and VLPs to create passive immunity was studied. This group concluded that VLPs were more effective than CLPs in inducing passive immunity.

Brief Summary Text (14):
Rotavirus proteins have even been used as immunological carrier complexes to facilitate the presentation of other epitopes to a subject. In one reference, VP6 was chosen as a carrier molecule for a particular antigen, based on the viral protein's ability to bind peptides. Sabara, et al., U.S. Pat. No. 5,374,426, issued Dec. 20, 1994, entitled, "Rotavirus Nucleocapsid Protein VP6 in Vaccine Compositions."

Brief Summary Text (15):
Exploiting another avenue, research has also been performed where a protective immune response was elicited using a DNA vaccine. Herrmann, et al., U.S. Pat. No. 5,620,896, issued Apr. 15, 1997, entitled, "DNA Vaccines Against Rotavirus Infections." While the results from this method are interesting, the degree of protection found in mice immunized with plasmids containing either the VP4, VP7 or VP6 genes in a murine retrovirus were very limited. Moreover, there are at least two reports of rotavirus DNA vaccines that failed to provide any protection to the immunized animal from rotaviral infection. Choi, et al., "Particle Bombardment-Mediated DNA Vaccination with Rotavirus VP6 Induces High Levels of Serum Rotavirus IgG but Fails to Protect Mice Against Challenge," Virology 232: 129-138 (1997). Choi et al. "Particle Bombardment-Mediated DNA Vaccination With Rotavirus VP4 or VP7 VP7 Induces High Levels of Serum IgG but Fails to Protect Mice Against Challenge," Virology 250:230-240 (1998).

Brief Summary Text (18):
The invention disclosed herein relates to compositions comprising various rotaviral proteins and methods of using these compositions to provide protection against rotaviral disease. One embodiment of the invention is a composition comprising a rotavirus VP6 protein or a fragment thereof, and an adjuvant in a pharmaceutical carrier, wherein said adjuvant is effective in generating a disease-reducing response to said VP6 protein.

Brief Summary Text (19):
Another embodiment of the invention encompasses a recombinant rotavirus fusion protein composition, comprising: a rotavirus subunit fusion protein or fragment thereof, a fusion protein partner in genetic association with said recombinant rotavirus subunit protein or fragment thereof, and an adjuvant in a pharmaceutical carrier, wherein said adjuvant is effective in stimulating a disease-reducing immunogenic response to said rotavirus fusion protein.

Brief Summary Text (20):
A full-length DNA copy of a gene encoding a recombinant rotavirus fusion protein, wherein said gene encoding said recombinant rotavirus protein comprising a rotavirus subunit protein or an immunogenic fragment thereof, and a fusion partner protein is contemplated in another embodiment of the invention disclosed herein.

Brief Summary Text (21):
A host cell comprising a DNA clone encoding recombinant rotavirus proteins is contemplated in another embodiment of the disclosed invention.

Drawing Description Text (2):
FIG. 1 shows some of the important features of pMAL-c2. Using this plasmid, recombinant plasmids were constructed which express chimeric proteins containing, the entire VP6, portions of VP6, the entire VP4 or a truncated form of VP7. pMAL-c2

contains a promoter sequence called Ptac which controls transcription of the fusion gene malE-lac Z.alpha.. The gene malE-lac Z.alpha. encodes a chimeric protein containing MBP and the a fragment of the enzyme .beta.-galactosidase. Rotavirus gene gene sequences were cloned into Xmn I, which is one of the multiple restriction sites present in the plasmid used for gene cloning. The created recombinant plasmids express chimeric proteins containing rotavirus proteins that are genetically fused with MBP. The precise site for Xmn I insertion is indicated by the arrow in FIG. 1. The same arrow also marks the exact position in the protein which can be enzymatically digested by Factor Xa in some, but not all, chimeric MBP proteins. pMAL-c2 also contains the lac Iq sequence which encodes a repressor protein that suppresses Ptac controlled transcription until IPTG is added. The plasmid also contains the colE1 origin of replication in E. coli and the ampicillin resistance gene that are typical features of many bacterial plasmids.

Drawing Description Text (4):
FIG. 3 shows evidence to establish that chimeric MBP::VP6 protein does not form structures that resemble rotavirus-like particles. Purified MBP::VP6 was put on the top of a sucrose gradient which was layered on top of a cesium chloride cushion. Rotavirus particles that were devoid of VP4 and VP7 were also layered on an identical sucrose gradient/cesium chloride cushion. By subjecting the protein and virus particles to a centrifugal force, the majority of the rotavirus particles traversed into fraction number 11 and 12 of the sucrose gradient and into fraction number 16 containing cesium chloride. In contrast, chimeric NBP::VP6 did not traverse into the gradient but remained in the top fractions (number 1 and 2). These results clearly demonstrated that the presence of MBP in the fusion protein does not allow MBP::VP6 to form any structures that resemble rotavirus particles.

Drawing Description Text (6):
FIG. 5 illustrates the region of VP6, which may assist in creating minimal subunit rotavirus vaccines. VP6, which consist of 397 amino acid residues, was delineated into four regions: regions A, B, C and D. The exact amino residues delineated by these regions were indicated. The gene sequences that encode regions A and B, B and C, or C and D were cloned into pMAL-c2 at the Xmn I site. The plasmids were given the names pMAL-c2/EDIM.sub.AB, pMAL-c2/EDIM.sub.BC and pMAL-c2/EDIM.sub.CD respectively.

Drawing Description Text (7):
FIG. 6 provides evidence that mice inoculated with chimeric MBP proteins containing regions A and B or containing regions C and D generated IgG which specifically recognized VP6. In these experiments, rotavirus particles were subjected to SDS-PAGE. The separated proteins were then transferred to nitrocellulose sheets. The sheets were cut into strips. Individual strips were incubated with a specific immune serum sample collected from mice inoculated with pMAL-c2/EDIM.sub.AB, pMAL-c2/EDIM.sub.BC or pMAL-c2/EDIM.sub.CD. While pMAL-c2/EDIM.sub.AB - and pMAL-c2/EDIM.sub.CD -inoculated mice generated anti-VP6 IgG, no specific IgG was detected by pMAL-c2/EDIM.sub.BC -immunized mice.

Drawing Description Text (8):
FIG. 7 further defines the CD region of VP6 in order to determine whether an even smaller minimal subunit rotavinis vaccine can be attained. The CD region was delineated into four regions: regions 1, 2, 3 and 4. The exact amino residues delineated by these regions were indicated. Recombinant plasmids were constructed using pMAL-c2 to harbor regions 1, 2, 3, and 4. The plasmids were given the names pMAL-c2/EDIM.sub.CD1, pMAL-c2/EDIM.sub.CD2, pMAL-c2/EDIM.sub.CD3 and pMAL-c2/EDIM.sub.CD4 respectively.

Detailed Description Text (3):
The vaccines of the present invention are composed of a native recombinant rotavirus protein or immunogenic fragment(s) thereof, a rotavirus fusion protein, or immunogenic fragment(s) thereof, an adjuvant, and a pharmaceutically acceptable

carrier. According to one embodiment of the present invention, a composition comprising a rotavirus protein or an immunogenic portion thereof is genetically associated with a fusion protein partner, and an adjuvant such as the A1 subunit, the B subunit of cholera toxin or E. coli heat-labile toxin present in a pharmaceutically acceptable carrier. This composition is administered to an individual in whom an immune response directed against the rotavirus subunit protein is sought and protection against rotavirus infection and disease is desired.

Detailed Description Text (6):
The rotavirus fusion proteins contemplated by the present invention are composed of a suitable fusion protein partner in genetic association with a rotavirus protein or immunogenic fragment thereof. The term in genetic association refers to a contiguous sequence of amino acids produced from a mRNA produced from a gene containing codons for the amino acids of the rotavirus protein and the fusion protein partner. A suitable fusion protein partner consists of a protein that will either enhance or at least not diminish the recombinant expression of the rotavirus fusion protein product when the two are in genetic association. Further, a suitable fusion protein partner may actively prevent the assembly of the rotavirus fusion proteins into multimeric forms after the rotavirus fusion protein has been expressed. For example, the fusion protein partner should prevent the formation of dimers, trimers or virus-like structures that might spontaneously form if the rotavirus protein were recombinantly expressed in the absence of the fusion protein partner. Still further, a suitable fusion partner will facilitate the purification of the chimeric rotavirus fusion protein. A representative list of suitable fusion protein partners includes maltose binding protein, poly-histidine segments capable of binding metal ions, inteine, antigens to which antibodies bind, S-Tag, glutathione-S-transferase, thioredoxin, .beta.-galactosidase, nonapeptide epitope tag from influenza hemagglutinin, a 11-amino acid epitope tag from vesicular stomatitis virus, a 12-amino acid epitope from the heavy chain of human Protein C, green fluorescent protein, cholera holo toxin or its B subunit, E. coli heat-labile holotoxin or its B subunit, CTA1-DD, streptavidin and dihydrofolate reductase.

Detailed Description Text (8):
In one embodiment of the present invention, rotavirus recombinant native or fusion proteins are mixed with an adjuvant such as a bacterial toxin. The bacterial toxin may be a cholera toxin. Alternatively, the rotavirus fusion protein may be mixed with the B subunit of cholera toxin (CTB). In another embodiment, an E. coli toxin may be mixed with the rotavirus fusion protein. For example, the rotavirus fusion protein may be mixed with E. coli heat-labile toxin (LT). The rotavirus fusion proteins of the present invention may be mixed with the B subunit of E. coli heat-labile toxin (LTB) to form a vaccine composition. Other adjuvants such as cholera toxin, labile toxin, tetanus toxin or toxoid, poly[di(carboxylatophenoxy) phosphazene] (PCPP), saponins Quil A, QS-7, and QS-21, RIBI (HAMILTON, Mont.), monophosphoryl lipid A, immunostimulating complexes (ISCOM), Syntax, Titer Max, M59, CpG, dsRNA, and CTA1-DD (the cholera toxin A1 subunit (CTA1) fused to a dimer of the Ig-binding D-region of Staphylococcus aureus protein A (DD)), are also contemplated.

Detailed Description Text (11):
The vaccine compositions of the invention contain conventional pharmaceutical carriers. Suitable carriers are well known to those of skill in the art. These vaccine compositions may be prepared in liquid unit dose forms. Other optional components, e.g., stabilizers, buffers, preservatives, excipients and the like may be readily selected by one of skill in the art. However, the compositions may be lyophilized and reconstituted by the individual administering the vaccine prior to administration of the dose. Alternatively, the vaccine compositions may be prepared in any manner appropriate for the chosen mode of administration, e.g., intranasal administration, oral administration, etc. The preparation of a pharmaceutically acceptable vaccine, having due regard to pH, isotonicity, stability and the like,

is within the skill of the art.

Detailed Description Text (14):
Vaccine compositions for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions or emulsions, the protein vaccine, and an adjuvant as described herein. The composition may be in the form of a liquid, a slurry, or a sterile solid which can be dissolved in a sterile injectable medium before use. The parenteral administration is preferably intramuscular. Intramuscular inoculation involves injection via a syringe into the muscle. This injection can be via a syringe or comparable means. The vaccine composition may contain a pharmaceutically acceptable carrier. Alternatively, the present vaccine compositions may be administered via a mucosal route, in a suitable dose, and in a liquid form. For oral administration, the vaccine composition can be administered in liquid, or solid form with a suitable carrier.

Detailed Description Text (21):
Construction of Recombinant pMAL-c2 Plasmids

Detailed Description Text (22):
Recombinant plasmids pMAL-c2/EDIM4, pMAL-c2/EDIM6 and pMAL-c2/LDIM7 were constructed constructed using pMAL-c2 (New England Biolabs, Beverly Mass.) by insertion of cDNAs encoding full length VP4 or VP6, or a truncated form of VP7 (TrVP7) of rotavirus strain EDIM (FIG. 1). cDNAs were synthesized by polymerase chain reaction (PCR) using the plasmids pGEM-3Z/EDIM4, pGEM-3Z/EDIM6 and pGEM-3Z/EDIM7 as templates templates and gene specific primers determined by nucleotide sequencing of the gene inserts. The nucleotide sequences have been deposited into GenBank nucleotide sequence database and assigned with the Accession Numbers AF039219, U65988 and AF039220 for VP4, VP6 and VP7 gene respectively.

Detailed Description Text (23):
The murine EDIM strain of rotavirus used for the construction of the pGEM recombinant plasmids was originally isolated from the stool of an infected mouse and and adapted to grow in cell culture by passage in MA-104 cells in the laboratory. A triply plaque-purified isolate of the ninth passage was used to infect MA-104 cells to yield stock virus for RNA purification. To generate cDNAs of rotavirus genes encoding strain VP4, VP6 and VP7, reverse transcription/polymerase chain reaction was carried out using purified genomic rotavirus RNA, a forward and a reverse primer obtained from the untranslatable regions of the gene. The cDNAs generated by RT/PCR were cloned into the Sma I site of the multiple cloning site of pGEM-3Z (Promega, Madison, Wis.). Ligation products were then transformed into E. coli. White transformants carrying recombinant plasmids were selected by growing cells on LB agar plates containing IPTG (0-5 mM) and X-gal. Plasmids from individual colonies were purified and were analyzed by nucleotide sequencing.

Detailed Description Text (24):
The cDNAs generated by PCR were inserted into the restriction site Xmn I of pMAL-c2, c2, placing the inserted sequences downstream from and in genetic association with the E. coli malE gene, which encodes maltose binding protein (MBP), resulting in the expression of MBP fusion protein. The plasmid utilized the strong "tac" promoter and the malE translation initiation signals to give high-level expression of the fusion protein. pMAL-c2 contains the factor Xa cleavage site that is located downstream from the malE sequence to enable cleavage of the heterologous protein from MBP. The plasmid conveyed ampicillin resistance to recombinant bacteria and a lacZ-alpha gene sequence for blue-to-white selection of recombinants with inserts.

Detailed Description Text (25):
Following ligation of cDNA and XmnI-digested pMAL-c2, recombinant pMAL-c2 plasmids were transformed into E. coli. White colonies of bacteria containing recombinant plasmids on an agar plate were then identified in the presence of IPTG and X-gal, and selected for further screening by PCR for gene identity and orientation.

Nucleotide sequencing was used to ultimately confirm the authenticity of the rotavirus gene sequence.

Detailed Description Text (34):
It has been shown that recombinant VP6 expressed by the bacculovirus expression system forms structures that resemble double-layered rotavirus particles when examined by electron microscopy. Purified MBP::VP6 fusion protein was analyzed by sucrose gradients to determine if these fusion proteins assembled into organized structures resembling virus particles that could be fractionated in a sucrose gradient. MBP::VP6 was subjected to centrifugation (SW 50, 35,000 g, 60 min) through a 4 ml sucrose gradient (20-50%) on a 1 ml cesium chloride cushion (60%). A total of 16, 300-.mu.l fractions were collected. Distribution of MBP::VP6 in the sucrose gradient and cesium chloride cushion was analyzed by Western blot analysis and distribution of virus particles was analyzed by silver nitrate staining of the SDS-gel (FIG. 3). The results showed that MBP::VP6 remained in the top 4 fractions of the gradient, while double-layered virus particles devoid of VP4 and VP7 were recovered from fraction #11 to #12 of the sucrose gradient and in the cesium chloride cushion (fraction #16). The difference in the distribution behavior of MBP::VP6 in the gradient indicated that the fusion protein does not form virus-like structures.

Detailed Description Text (44):
Stool specimens for Example 4 were thawed, homogenized and centrifuged (500 g, 10 min). For rotavirus antigen determination, 96-well EIA plates (Coming Costar Co., Coming, N.Y.) were coated overnight at 4.degree. C. with 100 .mu.l per well of either rabbit antibody to rotavirus (duplicate positive wells) or preimmune rabbit serum (duplicate negative wells). Plates were washed and 50 .mu.l of stool supernatant was added to duplicate wells coated with each antibody. After one hour incubation at 37.degree. C. on a rotation platform, plates were washed and 50 .mu.l normal goat serum (Vector Laboratory, Inc., Burlingame, Calif.) diluted 100-fold in phosphate-buffered saline containing 5% nonfat dry milk (PBS-M) was added for 15 minutes at room temperature. Fifty microliters of guinea pig antibody to rotavirus diluted 1:500 in PBS-M containing a 1:50 dilution of normal rabbit serum (DAKO, Carpinteria, Calif.) was added and incubated for 30 minutes. Plates were washed and 50 .mu.l of a 1:200 dilution of biotinylated goat anti-guinea pig IgG (Vector) in PBS-M containing a 1:50 dilution of normal rabbit serum was added and incubated 30 minutes. After washing plates, 50 .mu.l of a 1:100 dilution of peroxidase-conjugated avidin-biotin (Vector) in wash buffer was added and incubated 30 minutes. minutes. The plates were washed and 50 .mu.l substrate phenylenediamine with H.sub.2 O.sub.2 in citric acid-phosphate buffer) was added and incubated (room temperature) for 15 minutes. The reaction was stopped with 75 .mu.l of 1.0 M H.sub.2 SO.sub.4. The absorbance at 490 nm was measured and the net optical densities were determined by subtracting the average of the negative wells from the average of the positive wells. The specimen was considered positive for rotavirus if the average absorbance of the positive wells was greater than or equal to two times that of the negative wells and greater than or equal to 0.15.

Detailed Description Text (48):
Serum rotavirus IgA and IgG and rotavirus stool IgA were measured as follows. EIA plates (Corning Costar Co., Corning, N.Y.) were coated overnight at 4.degree. C. with anti-rotavirus rabbit IgG. After washing with phosphate buffered saline plus 0.05% Tween 20, 50 .mu.l of EDIM viral lysate or mock-infected cell lysate were each added to duplicate positive and duplicate negative wells and plates were incubated for one hour at 37.degree. C. on a rotation platform. After washing plates, 50 .mu.l of serial two-fold dilutions of pooled sera from EDIM infected mice assigned concentrations of 160,000 or 10,000 units/ml of rotavirus IgG or IgA, respectively, were added to duplicate wells coated with either EDIM-infected or uninfected MA104 cell lysates to generate a standard curve. Serial 10-fold dilutions of mouse sera to be tested were also added to duplicate wells of each lysate and incubated 1 hour. This was followed by sequential addition of biotin-

conjugated goat anti-mouse IgG or IgA (Sigma Chemical Co., St. Louis, Mo.), peroxidase-conjugated avidin-biotin (Vector Laboratories), and o-phenylenediamine substrate (Sigma Chemical Co.). Color development was stopped after fifteen minutes with 1 M H.sub.2 SO.sub.4 and the A.sub.490 was measured. Titers of rotavirus IgG or IgA, expressed as units/ml, were determined from the standard curve generated by subtraction of the average A.sub.490 values of the duplicate cell lysate wells from the average of the wells coated with EDIM lysate.

Detailed Description Text (53):
Stool specimens were thawed, homogenized and centrifuged (500 g, 10 min). For rotavirus antigen determination, 96-well EIA plates (Corning Costar Co.) were coated overnight at 4.degree. C. with 100 .mu.l/well of either rabbit antibody to rotavirus (duplicate positive wells) or preimmune rabbit serum (duplicate negative wells). Plates were washed and 50 .mu.l of stool supernatant or serial two-fold dilutions of purified preparation of double-layered EDIM particles (standard) was added to duplicate wells coated with each antibody. After incubation at 37.degree. C. for one hour on a rotation platform, plates were washed and 50 .mu.l normal goat serum (Vector) diluted 100-fold in phosphate-buffered saline containing 50% nonfat dry milk (PBS-M) was added for 15 minutes at room temperature. Fifty microliters of guinea pig antibody to rotavirus diluted 1:500 in PBS-M containing a 1:50 dilution of normal rabbit serum (DAKO) was added and incubated for 30 minutes. Plates were washed and 50 .mu.l of a 1:200 dilution of biotinylated goat anti-guinea pig IgG (Vector) in PBS-M containing a 1:50 dilution of normal rabbit serum was added and incubated 30 minutes. After washing plates, 50 .mu.l of a 1:100 dilution of peroxidase-conjugated avidin-biotin (Vector) in wash buffer was added and incubated 30 minutes. The plates were washed and 50 .mu.l substrate (o-phenylenediamine with H.sub.2 O.sub.2 in citric acid-phosphate buffer) was added and incubated (room temperature) for 15 minutes. The reaction was stopped with 75 .mu.l of 1.0 M H.sub.2 SO.sub.4. The absorbance at 490 nm was measured and the net optical densities were determined by subtracting the average of the negative wells from the average of the positive wells. Values obtained from a standard curve generated from the serially diluted double-layered particles were used to determine concentrations of rotavirus protein in each specimen. The limit of detection was 3 ng/ml.

Detailed Description Text (67):
To produce a minimal subunit vaccine while retaining the original protective efficacy, three plasmids, pMAL-c2/EDIM6.sub.AB, pMAL-c2/EDIM6.sub.BC and pMAL-c2/EDIM6.sub.CD, were constructed to express truncated forms of VP6, wherein the truncated forms of VP6 contain immunogenic fragments of a rotavirus protein. Recombinant plasmids pMAL-c2/EDIM6.sub.AB, pMAL-c2/EDIM6.sub.BC and pMAL-c2/EDIM6.sub.CD, containing truncated forms of VP6 were constructed using the same strategy that was used for the construction of pMAL-c2/EDIM6, as seen in Example 1. These plasmids expressed MBP::VP6.sub.AB containing amino acids 1 to 196, MBP::VP6.sub.BC containing amino acid 97 to 297 and MBP::VP6.sub.CD containing amino acids 197 to 397. (See FIG. 5). To construct these plasmids, cDNAs were synthesized by polymerase chain reaction (PCR) using pMAL-c2/EDIM6 (see Example 1) as the template. The gene specific primers used for construction and the regions of VP6 cloned are summarized in Table 6.

Detailed Description Text (68):
Once constructed, the plasmids encoding the truncated VP6 fragments were introduced into bacteria for protein expression. Recombinant bacteria containing pMAL-c2/EDIM6.sub.AB, PMAL-c2/EDIM6.sub.BC and PMAL-c2/EDIM6.sub.CD were grown as described in the examples above. Specifically, an overnight culture was grown (37.degree. C.; shaken at 215 rpm) in rich broth (tryptone, 10 gm; yeast extract, 5 gm; NaCl, 5 gm; glucose, 2 gm; and ampicillin, 100 mg per liter). On the following day, 10 ml of overnight culture for each vector were inoculated into 1 liter of rich broth. The culture was grown until the optical density reached .about.0.6 OD.sub.600. IPTG was added (0.3 mM) to induce expression of fusion protein. Growth was continued for 3 hours.

Detailed Description Text (76):
Serum rotavirus IgA and IgG and rotavirus stool IgA were measured by EIA. EIA plates (Coming Costar Co.) were coated overnight at 4.degree. C. with anti-rotavirus rabbit IgG. After washing with phosphate buffered saline plus 0.05% Tween 20, 50 .mu.l of EDIM viral lysate or mock-infected cell lysate were each added to duplicate positive and duplicate negative wells for one hour at 37.degree. C. on a rotation platform. After washing plates, 50 .mu.l of serial two-fold dilutions of pooled sera from EDIM infected mice assigned concentrations of 160,000 or 10,000 units/ml of rotavirus IgG or IgA, respectively, were added to duplicate wells coated with either EDIM-infected or uninfected MA104 cell lysates to generate a standard curve. Serial 10-fold dilutions of mouse sera to be tested were also added to duplicate wells of each lysate and incubated for 1 hour. This was followed by sequential addition of biotin-conjugated goat anti-mouse IgG or IgA (Sigma Chemical Co.), peroxidase-conjugated avidin-biotin (Vector Laboratories), and o-phenylenediamine substrate (Sigma Chemical Co.). Color development was stopped after fifteen minutes with 1 M H.sub.2 SO.sub.4 and the A.sub.490 was measured. Titers of rotavirus IgG or IgA, expressed as units/ml, were determined from a standard curve generated by subtraction of the average A.sub.490 values of the duplicate cell lysate wells from the average of the wells coated with EDIM lysate. For determination of stool rotavirus IgA, two stool pellets were collected into 0.5 ml of EBSS, homogenized, and centrifuged (1500 g, 5 min). Stool rotavirus IgA was then measured by the method described above.

Detailed Description Text (80):
Serum samples from mice immunized with vaccines were analyzed for rotavirus protein-specific antibodies by Western blot analyses. Cesium chloride gradient-purified rotavirus particles were subjected to SDS-polyacrylamide gel electrophoresis. Separated rotavirus proteins were blotted to a nitrocellulose sheet and cut into strips each of which contained 3 .mu.g of rotavirus proteins. The strips were blocked with 5% skim milk in Tris-HCl buffer (TBS, 50 mM Tris-HCl, pH 7.5, 0.9% NaCl). The strips were then incubated with antisera obtained from immunized mice. After washing with 0.1% Tween-20 in TBS, the strips were incubated with goat anti-mouse IgG conjugated to alkaline phosphatase (Life Technologies, Gaithersburg, Md.). The strips were washed with TBS and then incubated with 4-chloro-3-indolylphosphate and nitroblue tetrazolium (Life Technologies, Gaithersburg, Md.) to visualize bound antibodies.

Detailed Description Text (87):
Construction and Testing of Recombinant pMAL-c2 Plasmids Expressing Fragments of BMP::VP6.sub.CD Minimal Subunit Vaccine

Detailed Description Text (88):
Because the C-terminal 50% of VP6 CD region could induce the same level of protection as the entire VP6 protein, the protective domains of this 201-amino acid portion of VP6 were further mapped. Four overlapping regions of the C-terminal CD region of the VP6 gene were cloned into pMAL-c2 (FIG. 7). The specific primers that were used for construction of these plasmids are summarized in Table 9. As described described previously, cDNAs were inserted into the restriction site Xmn I of pMAL-c2, placing the inserted sequences downstream from the E. coli Mal E gene, which encodes maltose binding protein (MBP), resulting in expression of MBP fusion proteins. These plasmids expressed MBP::VP6.sub.CD1 containing amino acids 197 to 263, MBP::VP6.sub.CD2 containing amino acids 244 to 310, MBP::VP6.sub.CD3 containing amino acids 291 to 351, and MBP::VP6.sub.CD4 containing amino acids 332 to 397 (FIG. 8).

Detailed Description Text (89):
Recombinant plasmids were transformed into E. coli. White colonies of bacteria containing recombinant plasmids on agar plates were then identified in the presence of IPTG and X-gal, and selected for further screening by PCR for gene identity and

orientation. Recombinant bacteria were grown as described previously for pMAL-c2/EDIM6. An overnight culture was grown (37.degree. C.; shaken at 215 rpm) in rich broth (tryptone, 10 gm; yeast extract, 5 gm; NaCl, 5 gm; glucose, 2 gm; and ampicillin, 100 mg per liter). On the following day, 10 ml of overnight culture were were inoculated into 1 liter of rich broth. The culture was grown until the A.sub.600 reached .about.0.6. IPTG was added (0.3 mM) to induce expression of the fusion protein. Growth was then continued for 3 hours. Nucleotide sequencing was used to ultimately confirm the authenticity of the rotavirus gene sequences.

Detailed Description Text (105):
Studies using rotavirus particles for intranasal immunization have shown that CD8 cells are not needed for protection. Experiments were performed to determine whether immunization with VP6 can also mediate CD8-independent protection. To achieve this goal, .mu.Mt mice were depleted of CD8 by injection with anti-CD8 antibodies. These mice were then immunized intranasally with MBP::VP6 and LT(R192G) and challenged with EDIM, as discussed above. The results showed that these animals were equally protected from viral infection following immunization whether or not CD8 cells were present or removed at the time of challenge.

Detailed Description Text (108):
Based on results found with .mu.Mt mice, protection stimulated by VP6 was found not to be dependent on antibody production. Furthermore, protection following immunization with MBP::VP6 was not dependent on CD8 cells. This would leave CD4 cells as the most likely memory cells involved in protection. The importance of CD4 cells in protection following i.n. immunization using monoclonal antibody depletion as well as using genetically altered mice that lack CD4 cells was undertaken using the methods discussed above. In preliminary studies, it was found that i.n. immunization with double-layered rotavirus particles is much less protective in CD4-depleted or CD4 knock-out mice than in non-depleted or genetically normal mice. Similar results were obtained with CD4-depleted BALB/c mice immunized i.n. with the 6-14 peptide. It should be noted that peptide 6-14 discussed above provided nearly complete protection and this 14 amino acid peptide contains a known CD4 epitope.

Detailed Description Text (112):
To facilitate purification of a full length chimeric VP6, the plasmid pMAL-c2X (New England Biolabs, Beverly, Mass.) was used. The same construct can be used to express MBP::VP6::6Xhis or VP6::6Xhis. pMAL-c2X contains an Nde I site just 5' to the Mal E gene. This restriction site is one of 2 sites needed to delete the mal E sequence for the construction of pMAI-c2X/EDIM6-His6 that expresses the fusion protein 6his::VP6. The forward primer contains an Nde I site and a 5' terminal sequence of VP6 (nucleotides 4-21). The reverse sequence contains a stop codon (taa), 6 histidine-encoding codons and a 15-nucleotide VP6 carboxyl terminus sequence (Table 11). The newly added C-terminal 6Xhis fusion tag together with the N-terminal MBP enable the purification of the full-length VP6 using consecutive amylose resin and Talon (Palo Alto, Calif.) resin affinity chromatography. To create pMAL-c2X/EDIM6-6his, the recombinant plasmid was modified by digestion with Nde I and re-ligated to create the plasmid pc2X/EDIM6-6his. This plasmid expresses VP6::6Xhis that is devoid of MBP.

Detailed Description Text (120):
The construction of a recombinant rotavirus fusion protein using a fusion partner of 6 histidines rather than MBP is described below. Although MBP by itself did not induce protection or rotavirus-specific antibodies, it is not clear if it can modulate VP6-induced protective immunity. To determine whether MBP has any adjuvant effects on the protective efficacy of VP6, as well as to show the ability of other amino acid sequences to serve as fusion protein partners, a recombinant plasmid was constructed by first cloning VP6 into pMAL-c2X, as described above. This plasmid is identical to pMAL-c2 except that it contains a Nde I restriction site which, together with an engineered Nde I site (Table 11), allows the eventual deletion of the mal E sequence.

Detailed Description Text (121):
Characterization of the recombinant plasmid shows the authenticity of the coding region. The 6his::VP6 recombinant rotavirus fusion protein expresses well, compared to the expression levels of the other recombinant rotavirus fusion proteins. The presence of the 6his sequence may prevent the assembly of the recombinant rotavirus fusion protein into a multimeric form and facilitates the purification of the recombinant protein. The efficacy of 6his::VP6 to elicit a protective immune response from an individual immunized with a vaccine containing this protein is compared with that of MBP::VP6. The results of this comparison show that the two recombinant rotavirus fusion roteins are capable of eliciting protective immune responses.

Detailed Description Text (134):
It had already been shown that cholera toxin (CT), which is biologically and functionally related to LT, could replace LT(R192G) as adjuvant. The effectiveness of other adjuvants (PCPP, QS-21) have now been examined. Groups of mice were vaccinated with 2 i.n. immunizations (two weeks apart) with MBP::VP6 and adjuvant [PCPP, QS-21 or LT(R192G)] (Table 14). The adjuvant PCPP conferred an 80% reduction in shedding when administered intranasally with MBP::VP6 but it was not effective when given orally. In contrast, 59% and 43% reductions were observed when QS-21 was included with MBP::VP6 for oral and intranasal inoculation, respectively. As previously observed, LT(R192G) provided 99% protection when given with the vaccine intranasally but was less protective (85%) when administered orally. These results indicate that the choice of adjuvant and the route of mucosal inoculation both impact the efficacy of the VP6 vaccine.

Detailed Description Text (135):
To search for other effective adjuvants, nucleic acid adjuvants (CpG DNA from CpG ImmunoPharmaceuticals, Wellesley, Mass.), double-stranded RNA, and the cholera toxin A1-based gene fusion protein CTA1-DD (Agren, et al. J Immunol 162:2432-2440, 1999), will be tested for their effectiveness in stimulating VP6-induced protective immunity.

Detailed Description Text (142):
A VP6 protein from a human rotavirus strain is cloned and expressed as a fusion protein for development of a vaccine candidate to be tested in mice and humans. VP6 from human rotavirus strain CJN is cloned and its nucleotide sequence determined using standard techniques well known in the art. See Current Protocols in Molecular Biology, Eds. Ausubel, et al., John Wiley & Sons, Inc. The chimeric protein is tested in the mouse model to establish that a human VP6 protein from a group A virus can cross-protect against a heterologous group A (mouse EDIM) rotavirus. This human VP6 vaccine is tested in gnotobiotic pigs that have an immunological system similar to that of humans. This pig model allows for the testing of whether mouse or human VP6 can protect against human rotaviruses. The protein is used in subsequent human trials.

Detailed Description Text (151):
Individuals participating in this study are chosen who are healthy at the time of vaccination with either the test vaccine or the placebo. Test subjects are assigned to receive vaccine or placebo in a double-blind fashion using a block randomization scheme. An appropriate number of doses are administered over a given period of time, e.g., two months, to elicit an immune response.

Detailed Description Text (152):
Study participants are monitored throughout the following year to determine the incidence of rotavirus infection and the subsequent development of disease conditions. Participating subjects are contacted on a periodic basis during this period to inquire about symptoms of rotaviral disease, both in the test subject and in the subject's community. Local epidemiological surveillance records may also be

accessed.

Detailed Description Paragraph Table (6):
TABLE 6 Primers used to clone pMAL-c2/MBP.sub.AB, pMAL-c2/EDIM6.sub.BC and pMAL-c2/EDIM6.sub.CD Name of Fusion Plasmid Protein Primers pMAL-c2/MBP.sub.AB MBP::VP6.sub.AB Forward primer: atg gat gtg ctg tac tct atc SEQ ID NO. 1 Reverse primer: tca cga gta gtc gaa tcc tgc aac SEQ ID NO. 2 pMAL-c2/EDIM6.sub.BC MBP::VP6.sub.BC Forward primer: atg gat gaa atg atg cga gag tca SEQ ID NO. 3 Reverse primer: tca gaa tgg cgg tct cat caa ttg SEQ ID NO. 4 pMAL-c2/EDIM6.sub.CD MBP::VP6.sub.CD Forward primer: tgc gca att aat gct cca gct SEQ ID NO. 5 Reverse primer: tca ctt tac cag cat gct tct aat SEQ ID NO. 6

Detailed Description Paragraph Table (9):
TABLE 9 Primers used to clone pMAL-c2/EDIM6.sub.CD1, pMAL-c2/EDIM6.sub.CD2, pMAL-c2/EDIM6.sub.CD3 and pMal-c2c2/EDIM6.sub.CD4 Name of fusion Plasmid protein Primers pMAL-c2/EDIM6.sub.CD1 MBP::VP6.sub.CD1 Forward primer: atg gat gtg ctg tac tct atc SEQ. I.D. NO. 7 Reverse primer: tca gaa ctc aac ttc tac att att tgg SEQ. I.D. NO. 8 pMAL-c2/EDIM6.sub.CD2 MBP::VP6.sub.CD2 Forward primer: gca act aca tgg tac ttc aac cca SEQ. I.D. NO. 9 Reverse primer: tca att tgg gaa aag tgc agt cac tgc SEQ. I.D. NO. 10 pMAL-c2/EDIM6.sub.CD3 MBP::VP6.sub.CD3 Forward primer: tca ttt caa ttg atg aga ccg cca SEQ. I.D. NO. 11 Reverse primer: tca ttg tct gac tga cgt cac att ggc SEQ. I.D. NO. 12 pMAL-c2/EDIM6.sub.CD4 MBP::VP6.sub.CD4 Forward primer: gaa tca gtt ctc gcg gat gca agt SEQ. I.D. NO. 13 Reverse primer: tca ctt tac cag cat gct tct aat SEQ. I.D. NO. 14

Detailed Description Paragraph Table (17):
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Other Reference Publication (5):
McNeal, et al. (1998) Stimulation of local immunity and protection in mice by intramuscular immunization with triple-or double-layered rotavirus particles and QS-21. Virology 243. 158-166.

Other Reference Publication (7):
Agren, et al., "Adjuvanticity of the Cholera Toxin A1-Based Gene Fusion Protein, CTA1-DD, Is Critically Dependent on the ADP-Ribosyltransferase and Ig-Binding Activity", J. Immunology , 1999, 162; pp. 2432-2440.

Other Reference Publication (10):
Choi, et al., "Particle-Bombardment-Mediated DNA Vaccination with Rotavirus VP4 or VP7 Induces High Levels of Serum Rotavirus IgG but Fails to Protect Mice against Challenge", Virology 250, pp. 230-240, 1998.

Other Reference Publication (11):
Choi, et al., "Particle-Bombardment-Mediated DNA Vaccination with Rotavirus VP6 Induces High Levels of Serum Rotavirus IgG but Fails to Protect Mice against Challenge", Virology 232, pp. 129-138, 1997.

Other Reference Publication (12):
Fernandez, et al., "Passive Immunity to Bovine Rotavirus in Newborn Calves Fed Colostrum Supplements from Cows Immunized with Recombinant SA11 Rotavirus Core-Like Particle (CLP) or Virus-Like Particle (VLP) Vaccines", Vaccines, vol. 16, No. 5, pp. pp. 507-516, 1998.

Other Reference Publication (15):
O'Neal, et al., "Rotavirus Virus-Like Particles Administered Mucosally Induce Protective Immunity", J. Virology, 71(11), pp. 8707-8717, Nov. 1997.

Other Reference Publication (16):
O'Neal, et al., "Rotavirus 2/6 Viruslike Particles Administered Intranasally with Cholera Toxin, Escherichia coli Heat-Labile Toxin (LT), and LT-R192G Induce Protection from Rotavirus Challenge", J. Virology, 72(4), pp. 3390-3393, Apr. 1998.

CLAIMS:

1. A composition comprising a rotavirus VP6 protein or a COOH-terminal fragment thereof, and an adjuvant in a pharmaceutical carrier, wherein said adjuvant is effective in combination with said VP6 protein or COOH-terminal fragment thereof to generate a disease-reducing response to rotavirus infection in a mammal, wherein said VP6 protein or COOH-terminal fragment thereof is not assembled into a viral particle.

3. The composition of claim 1, wherein said adjuvant is selected from the group consisting of: cholera toxin (CT) and E. coli heat-labile toxin (LT).

4. The composition of claim 1, wherein said adjuvant is selected from the group consisting of PCPP, QS-21, QS-7, CTA1-DD, CpG DNA, and double-stranded RNA (dsRNA).

5. The composition of claim 1, wherein said composition is adapted for administration by a route selected from the group consisting of intramuscular administration, intranasal administration, oral administration, transdermal administration, and transmucosal administration.

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L32: Entry 46 of 52 File: USPT Oct 6, 1998

US-PAT-NO: 5817637
DOCUMENT-IDENTIFIER: US 5817637 A

TITLE: Genetic immunization

DATE-ISSUED: October 6, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weiner; David B.	Merion	PA		
Williams; William V.	Havertown	PA		
Wang; Bin	Havertown	PA		

US-CL-CURRENT: 514/44; 424/278.1, 435/975, 514/615, 514/818

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L32: Entry 46 of 52 File: USPT Oct 6, 1998

DOCUMENT-IDENTIFIER: US 5817637 A
TITLE: Genetic immunization

CLAIMS:

1. An pharmaceutical immunizing kit comprising:

a) a first inoculant comprising:

i) a pharmaceutically acceptable carrier or diluent; and,

ii) a first nucleic acid molecule comprising a nucleotide sequence that encodes at least one HIV protein operatively linked to regulatory sequences; wherein said nucleotide sequence is capable of being expressed in human cells;

b) a second inoculant comprising:

i) a pharmaceutically acceptable carrier or diluent; and,

ii) a second nucleic acid molecule comprising a nucleotide sequence that encodes at least one HIV protein operatively linked to regulatory sequences; wherein said nucleotide sequence is capable of being expressed in human cells;

wherein said first nucleic acid molecule is not identical to said second nucleic acid molecule and, taken together, said first nucleic acid molecule and said second nucleic acid molecule encode HIV proteins gag, pol and env; and

c) a third inoculant comprising bupivacaine.

2. A pharmaceutical composition comprising:

a) a compound selected from the group consisting of: bupivacaine, mepivacaine, lidocaine, procaine, carbocaine and methyl bupivacaine; and

b) a DNA molecule that comprises a DNA sequence that encodes an antigen; wherein said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence.

4. The pharmaceutical composition of claim 2 wherein said DNA molecule is a plasmid.

5. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a variable region of a T cell receptor.

6. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a pathogen antigen.

7. The pharmaceutical composition of claim 6 wherein said DNA sequence encodes an antigen from an intracellular pathogen.

14. The pharmaceutical composition of claim 2 wherein said DNA sequence encodes a hyperproliferative disease associated protein.

18. a method of immunizing an individual against an antigen comprising administering to tissue of said individual's body,

a) a compound selected from the group consisting of bupivacaine, mepivacaine, lidocaine, procaine, carbocaine and methyl bupivacaine, and

b) a DNA molecule that comprises a DNA sequence that encodes said antigen, said DNA sequence operatively linked to regulatory sequences which control the expression of said DNA sequence;

wherein said DNA molecule is taken up by cells, said DNA sequence is expressed in said cells and an immune response is generated against said antigen.

20. The method of claim 18 wherein said DNA molecule is a plasmid.

24. The method of claim 23 wherein said pathogen is HIV and said DNA molecule comprises a DNA sequence that encodes an HIV antigen.

29. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered subcutaneously.

30. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered intramuscularly, intraperitoneally, intravenously, intraarterially, intraoccularly, orally transdermally and/or by inhalation.

31. The method of claim 18 wherein said bupivacaine and a DNA molecule are administered intradermally.

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L45: Entry 6 of 9 File: USPT Mar 31, 1998

DOCUMENT-IDENTIFIER: US 5733726 A
** See image for Certificate of Correction **
TITLE: Cytotoxicity-based genetic selection system (TOXSEL)

CLAIMS:

1. A cytotoxicity-based genetic selection (TOXSEL) method for the positive selection of a molecule or a mutation that disrupts a specific protein--protein interaction between a first interacting protein and a second interacting protein, said method comprising the steps of:

(a) providing a host cell containing a detectable gene, said gene comprising:

(i) an upstream activation site for binding a DNA-binding domain;

(ii) a promoter; and

(iii) a reporter gene encoding a protein which is a toxin, said toxin being expressed upon transcriptional activation of said detectable gene, said transcriptional activation occurring when an amino acid sequence comprising a transcriptional activation domain is in sufficient proximity to said detectable gene;

(b) providing a first chimeric gene which is expressed in the host cell, said first chimeric gene comprising a DNA sequence encoding a first hybrid protein comprising:

(i) said DNA-binding domain recognizing said upstream activation site on said detectable gene in the host cell; and

(ii) a first interacting protein which interacts specifically with a second interacting protein;

(c) providing a second chimeric gene which is expressed in the host cell, said second chimeric gene comprising a DNA sequence encoding a second hybrid protein comprising:

(i) said transcriptional activation domain; and

(ii) said second interacting protein which interacts specifically with said first interacting protein;

(d) introducing said first chimeric gene and said second chimeric gene into the host cell;

(e) subjecting the host cell to conditions allowing said first hybrid protein and second hybrid protein to be expressed in quantities sufficient to allow specific interaction between said first interacting protein and said second interacting

protein resulting in the positioning of said transcriptional activation domain in sufficient proximity to said detectable gene so that said detectable gene is transcriptionally activated to cause expression of said toxin reporter gene, resulting in death of the host cell; or

(f) introducing into the host cell a molecule to be tested for its capability to disrupt said interaction between said first interacting protein and said second interacting protein; and

(g) subjecting the host cell to conditions

(i) allowing said first hybrid protein and said second hybrid protein to be expressed in quantities sufficient to allow specific interaction between said first interacting protein and said second interacting protein such that transcription is activated, said toxin is expressed and said host cell dies if said test molecule is not capable of disrupting said protein--protein interaction, and

(ii) allowing said test molecule, if it is capable of doing so, to disrupt said interaction between said first interacting protein and said second interacting protein, thereby disrupting transcriptional activation of said detectable gene, which disrupts expression of said toxin reporter gene, and results in survival of the host cell.

3. The method according to claim 1 wherein said toxin is selected from the group consisting of diphtheria toxin, ricin, exotoxin A of Pseudomonas aeruginosa and Shiga toxin.

5. The method according to claim 1 wherein said first interacting protein or second interacting protein is selected from the group consisting of bacterial protein, viral protein, oncogene/proto-oncogene-encoded protein, growth factor, receptor protein, regulatory protein and enzyme.

9. The method according to claim 2 wherein said host cell is a yeast cell or a mammalian cell.

10. The method according to claim 3 wherein said toxin is a diphtheria toxin catalytic A fragment or a toxic mutant thereof.

11. The method according to claim 4 wherein said transcriptional activators are bacterial, viral or eukaryotic.

13. A kit for the positive selection of a molecule which disrupts a specific protein--protein interaction between a first interacting protein and a second interacting protein, comprising a container, three vectors and a host cell,

said first vector comprising a promoter transcribing a first chimeric gene comprising a DNA sequence encoding a first hybrid protein including a DNA-binding domain and a first interacting protein,

said second vector comprising a promoter transcribing a second chimeric gene comprising a DNA sequence encoding a second hybrid protein including a transcriptional activation domain and a second interacting protein,

said host cell comprising a third vector comprising a detectable gene, said detectable gene comprising a binding site for said DNA-binding domain of said first hybrid protein, a promoter, a reporter gene encoding a protein which is a toxin, said DNA-binding domain, said promoter and said reporter gene so positioned that activation of said detectable gene occurs when said transcriptional activation domain of said second hybrid protein is in sufficient proximity to said detectable gene,

said container comprising means for housing incubation of said host cell with said first and second vectors under conditions allowing either specific interaction between said first interacting protein and said second interacting protein or disruption of said protein--protein interaction in the presence of a test molecule which disrupts said protein--protein interaction.

16. The kit of claim 15 wherein said transcriptional activators are bacterial, viral viral or eukaryotic.

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L20: Entry 2 of 32 File: USPT Apr 13, 2004

US-PAT-NO: 6720001
DOCUMENT-IDENTIFIER: US 6720001 B2

TITLE: Emulsion compositions for polyfunctional active ingredients

DATE-ISSUED: April 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chen; Feng-Jing	Salt Lake City	UT		
Patel; Mahesh V.	Salt Lake City	UT		

US-CL-CURRENT: 424/455; 424/400, 424/450, 424/456

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L20: Entry 2 of 32 File: USPT Apr 13, 2004

DOCUMENT-IDENTIFIER: US 6720001 B2
TITLE: Emulsion compositions for polyfunctional active ingredients

Brief Summary Text (2):
The present invention relates to pharmaceutical delivery systems, and in particular to oil-in-water pharmaceutical emulsions for the improved delivery of polyfunctional active ingredients, such as pharmaceuticals, cosmeceuticals, nutritive agents, and diagnostic agents.

Brief Summary Text (9):
Several formulations have been developed based on medium chain triglyceride ("MCT") oil phases, rather than (or in addition to) the more traditional long chain triglyceride ("LCT") oil phases. U.S. Pat. No. 5,660,858 to Parikh et al. discloses cyclosporin oil-in-water emulsions with a synthetic MCT having predominantly C.sub.8 -C.sub.12 fatty acid chains. U.S. Pat. No. 5,364,632 to Benita et al. discloses an oil-in-water emulsion of a lipophilic drug having an MCT oil carrier and a combination of ionic and non-ionic surfactants.

Brief Summary Text (66):
Polyfunctional active ingredients can be, for example, analgesics and anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, anti-asthma agents, anti-bacterial agents, anti-viral agents, anti-coagulants, anti-depressants, anti-diabetics, anti-epileptics, anti-fungal agents, anti-gout agents, anti-hypertensive agents, anti-malarials, anti-migraine agents, anti-muscarinic agents, anti-neoplastic agents and immunosuppressants, anti-protozoal agents, anti-thyroid agents, anti-tussives, anxiolytic, sedatives, hypnotics and neuroleptics, .beta.-Blockers, cardiac inotropic agents, corticosteroids, diuretics, anti-parkinsonian agents, gastro-intestinal agents, histamine H,-receptor antagonists, keratolytics, lipid regulating agents, muscle relaxants, anti-anginal agents, sex hormones and stimulants,. The polyfunctional active ingredient can also be a cytokine, a peptidomimetic, a protein, a peptide, a toxoid, a serum, an antibody, a vaccine, a nucleoside, a nucleotide, a portion of genetic material, a nucleic acid, DNA, RNA, an oligodeoxynucleotide, or an oligonucleotide.

Brief Summary Text (67):
Examples of suitable polyfunctional active ingredients include acarbose; acyclovir; acetyl cysteine; acetylcholine chloride; alatrofloxacin; alendronate; alglucerase; amantadine hydrochloride; ambenomium; amifostine; amiloride hydrochloride; aminocaproic acid; amphotericin B; antihemophilic factor (human); antihemophilic factor (porcine); antihemophilic factor (recombinant); aprotinin; asparaginase; atenolol; atracurium besylate; atrophine; azithromycin; aztrconam; BCG vaccine; bacitracin; becaplermin; belladona; bepridil hydrochloride; bleomycin sulfate; calcitonin human; calcitonin salmon; carboplatin; capecitabine; capreomycin sulfate; cefamandole nafate; cefazolin sodium; cefepime hydrochloride: cefixime: cefonicid sodium; cefoperazone; cefoteran disodium: cefotaxime; cefoxitin sodium; ceftizoxime; cefhiaxone; cefuroxime axetil; cephalexin; cephapirin sodium; cholera vaccine; chorionic gonadotropin; cidofovir; cisplatin; cladribine; clidinium bromide; clindamycin and clindamycin; ciprofloxacin; clodronate; colistimethate sodium; colistin sulfate; corticotropin; cosyntropin; cromolyn sodium; cytarabine;

dalteperin sodium; danaparoid; deferoxamine; denileukin diftitox; desmopressin; diatrizoate meglumine and diatrizoate sodium; dicyclomine; didanosine; dirithromycin; dopamine hydrochloride; dornase alpha; doxacurium chloride; doxorubicin; clidonate disodium; enalaprilat; enkephalin; enoxacin; enoxaparin sodium; ephedrine; epinephrine; epoetin alpha; erythromycin; esmolol hydrochloride; factor IX; famcilovir; fludarabine; fluoxetine; foscarnet sodium; ganciclovir; granulocyte colony stimulating factor; granulocyte-macrophage stimulating factor; recombinant human growth hormone; bovine growth hormone; gentamycin; glucagon; glycopyrolate; gonadotropin releasing hormone and synthetic analogs thereof; gonadorelin; grepafloxacin; haemophilus B conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus vaccine inactivated; heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin porcine; insulin NPII; insulin aspart; insulin glargine; insulin detemir; interferon alpha; interferon beta; ipratropium bromide; isophosphamide; Japanese encephalitis virus vaccine; lamivudine; leucovorin calcium leuprolide acetate; levofloxacin; lincomycin and lincomycin derivatives; lobucavir; lomefloxacin; loracarbef, mannitol; measles virus vaccine; meningococcal vaccine; menotropins; mepenzolate bromide; mesalamine; methenamine; methotrexate; methscopolamine; metformin hydrochloride; metoprolol; mezlocillin sodium; mivacurium mivacurium chloride; mumps viral vaccine; nedocromil sodium; neostigmine bromide; neostigmine methyl sulfate; neurontin; norfloxacin; octreotide acetate; ofloxacin; olpadronate; oxytocin; pamidronate disodium; pancuronium bromide; paroxetine; pefloxacin; pentamidine isethionate; pentostatin; pentoxifylline; penciclovir; pentagastrin; phentolamine mesylate; phenylalanine; physostigmine salicylate; plague plague vaccine; piperacillin sodium; platelet derived growth factor-human; pneumococcal vaccine polyvalent; poliovirus vaccine inactivated; poliovirus vaccine live (OPV); polymyxin B sulfate; pralidoxime chloride; pramlintide; pregabalin; propafenone; propantheline bromide; pyridostigmine bromide; rabies vaccine; risedronate; ribavirin; rimantadine hydrochloride; rotavirus vaccine; salmeterol xinafoate; sincalide; small pox vaccine; sotalol; somatostatin; sparfloxacin; spectinomycin; stavudine; streptokinase; streptozocin; suxamethonium chloride; tacrine hydrochloride; terbutaline sulfate; thiotepa; ticarcillin; tiludronate; timolol; tissue type plasminogen activator; TNFR:Fe; TNK-tPA; trandolapril; trimetrexate gluconate; trospectomycin; trovafloxacin; tubocurarine chloride; tumor necrosis factor; typhoid vaccine live; urea; urokinase; vancomycin; valacyclovir; valsartan; varicella virus vaccine live; vasopressin; vecuronium bromide; vinblastine; vincristine; vinorelbine; warfarin sodium; yellow fever vaccine; zalcitabine; zanamivir; zolodronate; and zidovudine.

Brief Summary Text (70):
The emulsions of the present invention can be produced by methods known in the art for forming emulsions, and examples of particular methods are shown in the Examples herein. It should be understood that formulation factors such as active characteristics, packaging, excipient purity and sourcing, and processing factors, such as sequence or method of excipient/drug addition, energy input, and sterilization cycle factors affect the commercial viability or therapeutic benefit of any given emulsion product.

Brief Summary Text (72):
The oil solution is then mixed with an aqueous solution at an appropriate temperature and for a sufficient amount of time to fully emulsify the oil. The mean diameter of the resulting coarse emulsion is preferably less than 20 microns. The resulting mixture is further homogenized at a desired pressure in batch-wise or continuous cycles until the desired particle size is obtained, typically a submicron submicron particle size. Several high pressure homogenizers are available for this process, including EmulsiFlex (Avestin), microfluidizer (Microfluidics), and Rannie homogenizer (APV). The resulting emulsion can be further pH adjusted and heat-, filter-, or radiation-sterilized.

Brief Summary Text (74):

Preferred emulsions can have a mean particle diameter of less than about 5 .mu.m, preferably less than about 2 .mu.m, more preferably less than about 1 .mu.m, still more preferably less than about 0.5 .mu.m, and most preferably less than about 0.3 .mu.m. Particle size can be determined by conventional methods, such as by measurement with a particle size analyzer.

Brief Summary Text (91):
The present invention is also directed to dosage forms of any of the pharmaceutical emulsions described herein. The dosage form can be the pharmaceutical emulsion processed by lyophilization, encapsulation, extrusion, homogenization, sonication, melting, solubilizing, evaporation, mixing, coating, size reduction, spraying, sterilization, filtration, irradiation, or a combination thereof It should be appreciated that the ability of the pharmaceutical composition to be processed by particular processing methods may depend upon the proper choice and sequence of processing steps, as is known to those skilled in the art.

Brief Summary Text (102):
6. Safer additives. Polarity modifiers, such as mono- and diglycerides, especially of longer chain fatty acids, are expected to be less "leaky" from the oil particles upon injection, or less prone to rapid debinding/desorption from the lipoprotein blood fraction, due to the high hydrophobicity. Thus, these additives have lower free monomer activity in the blood, leading to superior bioacceptability of additives relative to high HLB surfactants.

Brief Summary Text (105):
The stability of the emulsion also can be improved by charge repulsion imparted by the incorporation of appropriate charged or charge-inducing emulsifiers or polarity modifiers to the emulsion formulation. Typically, a negatively charged phospholipid, such as DMPG, can decrease the zeta potential of the emulsion particles (or increase the surface charge) to about -30 to -60 mV, which would provide significant repulsion forces to prevent the emulsion particles from agglomeration, fusion, etc. Similarly, polyethoxylated surfactants, amphiphilic synthetic polymers and certain amphiphilic proteins/peptides, can form shields surrounding emulsion particles to prevent the close contact of two or more particles through steric hindrance. The enormous van der Waals forces will repel particles when the shields of different particles are approaching to a close distance.

Brief Summary Text (107):
9. More favorable binding. The emulsions composition of the present invention, through the unique combination of the polarity modifiers as well as the emulsifiers, possess unique size, surface, and polarity characteristics to alter the biodistribution and clearance of the emulsion particles as well as the pharmacokinetic and pharmacological profile of the polyfunctional actives either incorporated in the emulsion formulation or co-administered with the emulsion. These altered characteristics of the actives and the emulsion oil droplets are potentially useful in targeting the actives to a variety of cells, tissue, and organs. Such compositions can be injected directly to a target organ or a tumor to improve the therapeutic performance of the active. This can potentially lead to the improvement of efficacies of current indications as well as to the discovery of new indications with existing actives. Since the polyfunctional active can be delivered more effectively to the site of action, the undesirable exposure of the active to other local tissues or systemic circulation could be reduced. This will lead to the reduction of systemic or local toxicity.

Brief Summary Text (110):
In addition, the emulsion formulations of the present invention can further include a ligand or receptor on the surface of the emulsion particles to promote more specific recognition between the emulsion particles and the intended sites for targeted delivery. For example, an antibody can be grafted to the surface of the

emulsion particles to specifically target cancerous cells expressing particular antigens recognized by the antibody. Through such antibody-antigen interaction, a given anticancer drug can be more effectively delivered to the site of action by the emulsion formulation to alleviate potential dose-limiting systemic toxicity.

Brief Summary Text (114):
13. Benefits in delivery, storage and dosing. Unlike solid particulate delivery for pulmonary, nasal or buccal delivery, the present compositions offer more reproducible, bioacceptable emulsion particles of size more amenable to better absorption from the absorption site, consistent dosing, and better storage properties.

Detailed Description Text (5):
The emulsion was prepared as follows. Captex 810D and safflower oil were mixed to form a homogeneous oil. Cyclosporin A and BHT were added to the oil and dissolved at room temperature. Egg phospholipid and DMPG were added to the oil mixture and dispersed in the oil phase. The oil phase was heated to 60.degree.-70.degree. C., then added to an aqueous phase of water, glycerol and EDTA. The mixture was then mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 300 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (9):
The emulsion was prepared as follows. Captex 810D, Eastman 9-45 and Peceol were mixed to form a homogeneous oil. Cyclosporin A was added to the oil and dissolved at room temperature. Egg phospholipid was added to the oil mixture and dispersed in the oil phase. The oil phase was heated to 60.degree.-70.degree. C., then added to an aqueous phase of water, glycerol and EDTA. The mixture was then mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 200 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (13):
The emulsion was prepared as follows. Safflower oil and Eastman 9-45 were mixed to form a homogeneous oil. Cyclosporin A and BHT were added to the oil and dissolved at room temperature. Egg phospholipid was dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 300 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (17):
The emulsion was prepared as follows. Soybean oil, Eastman 9-45 and Peceol were mixed to form a homogeneous oil. Progesterone and BHT were added to the oil and dissolved at room temperature. Egg phospholipid was added to the oil mixture and dispersed in the oil phase. The oil mixture was heated to 60.degree. C.-70.degree. C., then added to an aqueous phase containing water, glycerol and EDTA. The mixture was mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 200 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (21):
The emulsion was prepared as follows. Captex 810D, Captex GTO and Capmul MCM were

mixed to form a homogeneous oil. Progesterone and BHT were added to the oil and dissolved at room temperature. Egg phospholipid and DMPG were dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree. C.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 150 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (25):
The emulsion was prepared as follows Safflower oil, triethylamine, Eastman 9-45 and Imwitor 988 were mixed to form a homogeneous oil Tretinoin and BHT were added to the oil and dissolved at room temperature. Egg phospholipid was dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree. C.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 100 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (26):
The Example was repeated, but after combining the oil and aqueous phases, the mixture was sonicated at room temperature using a Branson sonifier. The pooled sonified material was then high pressure homogenized as previously described. The resulting emulsion had mean particle diameter of less than 80 nm.

Detailed Description Text (30):
The emulsion was prepared as follows. Captex 810D, triethylamine, Peceol and Capmul MCM were mixed to form a homogeneous oil. Tretinoin and BHT were added to the oil and dissolved at room temperature. Egg phospholipid was dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree. C.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 100 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (39):
The emulsion was prepared as follows. Captex 810D, soybean oil and Labrafil M2125 CS were mixed to form a homogeneous oil. Egg phospholipid was dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree. C.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 150 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (43):
The emulsion was prepared as follows. Captex 810D, safflower oil and Lauroglycol FCC were mixed to form a homogeneous oil. Egg phospholipid was dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree. C.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 100 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (47):
The emulsion was prepared as follows. Safflower oil and Plurol Oleique CC497 were mixed to form a homogeneous oil. Egg phospholipid and DMPG were dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree. C.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 100 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

Detailed Description Text (51):
The emulsion was prepared as follows. Soybean oil and Kessco PEG 400DO were mixed to form a homogeneous oil. Egg phospholipid was dispersed in an aqueous phase containing water, glycerol and EDTA. Both the oil phase and the aqueous phase were heated to 60.degree. C.-70.degree. C., then combined and mixed well using an UltraTurrax homogenizer (IKA). The mixture was further high pressure homogenized by a microfluidizer (Microfluidics) at a pressure of 16,000 psi for 10 cycles in succession. The resulting emulsion had a mean particle diameter of less than 100 nm, as measured by a Nicomp particle size analyzer (Particle Size Systems, Inc.).

CLAIMS:

1. A stabilized pharmaceutical oil-in-water emulsion for delivery of a polyfunctional drug, wherein the emulsion has a mean particle diameter of less than about 5 .mu.m and consists essentially of: (a) a therapeutically effective amount of a polyfunctional drug selected from the group consisting of analgesics, anti-inflammatory agents, anthelmintics, antiarrhythimic agents, anti-asthma agents, anti-bacterial agents, anti-viral agents, anti-coagulants, anti-depressants, anti-diabetic agents, anti-epileptic agents, anti-fungal agents, anti-gout agents, anti-hypertensive agents, anti-malarials, anti-migraine agents, anti-muscarinic agents, anti-neoplostic agents, immunosuppressants, anti-protozoal agents, anti-thyroid agents, anti-tussives, anxiolytics, sedatives, hypnotics, neuroleptic agents, .beta.-blockers, cardiac inotropic agents, corticosteroids, diuretics, anti-parkinsonism agents, gastrointestinal agents, histamine receptor antagonists, keratolytics, lipid regulating agents, muscle relaxants, anti-anginal agents, sex hormones, stimulants, cytokines, peptidomimetics, proteins, peptides, toxoids, antibodies, vaccines, nucleosides, nucleotides, nucleic acids, DNA, RNA, oligonucleotides, oligodeoxynucleotides, and combinations thereof; (b) an aqueous phase; (c) an oil phase consisting essentially of (i) a mixture of structured triglycerides having one medium chain fatty acid (MCFA) group and at least one long chain fatty acid (LCFA) groups, wherein the total amount of fatty groups of the oil phase having a carbon chain length of from 6-12 carbons atoms is less than about 30% and the total amount of fatty acid groups of the oil phase having a carbon chain length of greater than 12 carbon atoms is greater than 10% by weight, based on the total weight of the fatty acid groups of the oil phase, and (ii) a polarity modifier effective to facilitate the incorporation of the polyfunctional drug into the emulsion, wherein the polarity modifier is selected from the group consisting of inorganic acids and inorganic; and (d) an amount of an emulsifier effective to provide a stabilized emulsion suitable for parenteral administration, wherein the emulsifier is selected from the group consisting of ceramides, mixed chain phospholipids, cationic lipids, oligolipids, phospholipids, carnitines, sphingosines, sphingomyelins, glycolipids, lipoproteins, apoproteins, amphiphilic proteins, amphiphilic peptides, amphiphilic synthetic polymers, and combinations thereof.

15. The pharmaceutical emulsion of claim 1, wherein the polyfunctional drug is selected from the group consisting of acarbose; acyclovir; acetyl cysteine; acetylcholine chloride; alatrofloxacin; alendronate; alglucerase; amantadine

hydrochloride; ambenomium; amifostine; amiloride hydrochloride; aminocaproic acid; amphotericin B; antihemophilic factor (human); antihemophilic factor (poreine); antihemophilic factor (recombinant); aprotinin; asparaginase; atenolol; atracurium besylate; atropine; azithromycin; aztreonam; BCG vaccine; bacitracin; becaplemin; belladona; bepridil hydrochloride; bleomycin sulfate; calcitonin human; calcitonin salmon; carboplatin; capecitabine; capreomycin sulfate; cefamandole nafate; cefazolin sodium; cefepime hydrochloride; cefixime; cefonicid sodium; cefoperazone; cefotetan disodium; cefotaxime; cefoxitin sodium; ceftizoxime; ceftriaxone; cefuroxime axetil; cephalexin; cephapirin sodium; cholera vaccine; chorionic gonadotropin; cidofovir; cisplatin; cladribine; clidinium bromide; clindamycin and ciprofloxacin; clodronate; colistimethate sodium; colistin sulfate; corticotropin; cosyntropin; cromolyn sodium; cytarabine; dalteperin sodium; danaparoid; deferoxamine; denileukin diftitox; desmopressin; diatrizoate meglumine and diatrizoate sodium; dicyclomine; didanosine; dirithromycin; dopamine hydrochloride; dornase alpha; doxacurium chloride; doxorubicin; etidronate disodium; enalaprilat; enkephalin; enoxacin; enoxaparin sodium; ephedrine; epinephrine; epoetin alpha; erythromycin; esmolol hydrochloride; factor IX; famciclovir; fludarabine; fluoxetine; foscarnet sodium; ganciclovir; granulocyte colony stimulating factor; granulocyte-macrophage stimulating factor; recombinant human growth hormones; bovine growth hormones; gentamycin; glucagon; glycopyrolate; gonadotropin releasing hormone and synthetic analogs thereof, gonadorelin; grepafloxacin; haemophilus B conjugate vaccine; Hepatitis A virus vaccine inactivated; Hepatitis B virus vaccine inactivated; heparin sodium; indinavir sulfate; influenza virus vaccine; interleukin-2; interleukin-3; insulin-human; insulin lispro; insulin porcine; insulin NPH; insulin aspart; insulin glargine; insulin detemir; interferon alpha; interferon beta; ipratropium bromide; isophosphamide; Japanese encephalitis virus vaccine; lamivudine; leucovorin calcium; leuprolide acetate; levofloxacin; lincomycin and lincomycin derivatives; lobucavir; lomefloxacin; loracarbef, mannitol; measles virus vaccine; meningococcal vaccine; menotropins; mepenzolate bromide; mesalamine; methenamine; methotrexate; methscopolamine; metformin hydrochloride; metoprolol; mezlocillin sodium; mivacurium chloride; mumps viral vaccine; nedocromil sodium; neostigmine bromide; neostigmine methyl sulfate; neurontin; norfloxacin; oetreotide acetate; ofloxacin; olpadronate; oxytocin; pamidronate disodium; pancuronium bromide; paroxetine; pefloxacin; pentamidine isethionate; pentostatin; pentoxifylline; penciclovir, pentagastrin; phentolamine mesylate; phenylalanine; physostigmine salicylate; plague vaccine; piperacillin sodium; platelet derived growth factor-human; pneumococcal vaccine polyvalent; poliovirus vaccine inactivated; poliovirus vaccine live (OPV); polymyxin B sulfate; pralidoxime chloride; pramlintide; pregabalin; propaferone; propantheline bromide; pyridostigmine bromide; rabies vaccine; risedronate; ribavirin; rimantadine hydrochloride; rotavirus vaccine; salmeterol xinafoate; sincalide; small pox vaccine; sotalol; somatostatin; sparfloxacin; spectinomycin; stavudine; streptokinase; streptozocin; suxamethonium chloride; tacrine hydrochloride; terbutaline sulfate; thiotepa; ticarcillin; tiludronate; timolol; tissue type plasminogen activator; TNFR:Fc; TNK-tPA; trandolapril; trimetrexate gluconate; trospectomycin; trovafloxacin; tubocurarine chloride; tumor necrosis factor; typhoid vaccine live; urea; urokinase; vancomycin; valacyclovir; valsarian; varicella virus vaccine live; vasopressin and vasopressin; vecuronium bromide; vinblastine; vincristine; vinorelbine; warfarin sodium; yellow fever vaccine; zalcitabine; zanamivir; zoledronate; zidovudine; pharmacoutically acceptable salts, isomers, and derivative thereof; and mixtures thereof.

20. The dosage form of claim 17, wherein the dosage form is adapted for parental, enteral, ocular, nasal, sublingual, buccal, topical, intra-cervical, rectal, intramuscular, intra-dermal, pulmonary, transmucosal, intra-thecal, intravenous, intra-arterial, epidural, intra-cavity, intra-organ transdermal, intra-lymphatic, intra-cranular, or intra-lumoral administration, or a combination thereof.

21. The pharmaceutical emulsion of claim 1, having a mean particle diameter of less than about 5 .mu.m.

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L47: Entry 13 of 52 File: USPT Jul 23, 2002

DOCUMENT-IDENTIFIER: US 6423513 B1
TITLE: Polynucleotides encoding protease-activatable pseudomonas exotoxin a-like proproteins

CLAIMS:

1. A recombinant polynucleotide comprising a nucleotide sequence encoding a protease-activatable Pseudomonas exotoxin A-like ("PE-like") proprotein comprising: (a) a cell recognition domain of between 10 and 1500 amino acids that binds to an exterior surface of a targeted cell; (b) a modified PE translocation domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 280 to 344 of SEQ ID NO:2 and which effects translocation to a cell cytosol upon proteolytic cleavage, wherein the translocation domain comprises a cysteine-cysteine loop that comprises a protease activatable sequence cleavable by a protease and wherein the protease activatable sequence is refractory to cleavage by furin when incubated with furin at a 1:10 enzyme:substrate molar ratio at 25.degree. C. for 16 hours; (c) a cytotoxicity domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 400 to 613 of SEQ ID NO:2, the cytotoxicity domain having ADP-ribosylating activity; and (d) an endoplasmic reticulum ("ER") retention sequence.

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L6: Entry 21 of 23 File: USPT Apr 26, 1994

DOCUMENT-IDENTIFIER: US 5306631 A
TITLE: Compositions and method for inhibition of HIV production

CLAIMS:

1. A method for the selective killing of an HIV-infected cell, said method comprising the step of:

(a) introducing into said cell a recombinant DNA molecule comprising an HIV-regulated chimeric diphtheria toxin fragment A gene, wherein said diphtheria toxin fragment A gene is expressed under the regulatory control of HIV cis-acting regulatory sequences and trans-acting factors,

wherein the expression of said HIV-regulated chimeric diphtheria toxin fragment A gene is activated by HIV trans-acting factors present in said HIV-infected cell, thus killing said HIV-infected cell which has incorporated said recombinant DNA molecule after activation by the HIV trans-acting factors.

8. A method for selective killing of a stably transformed cell line after infection with HIV, wherein said cell line is stably transformed with a recombinant DNA molecule comprising an HIV-regulated chimeric diphtheria toxin fragment A gene, wherein said diphtheria toxin fragment A gene is expressed under the regulatory control of HIV cis-acting regulatory sequences and HIV trans-acting factors, and wherein the expression of said chimeric diphtheria toxin fragment A gene is activated in the presence of HIV trans-acting factors, whereby when said cell line is infected with HIV, HIV trans-acting factors activate the expression of said chimeric diphtheria toxin fragment A gene, thus killing the HIV-infected, stably transformed cell.

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L6: Entry 21 of 23 File: USPT Apr 26, 1994

US-PAT-NO: 5306631
DOCUMENT-IDENTIFIER: US 5306631 A

TITLE: Compositions and method for inhibition of HIV production

DATE-ISSUED: April 26, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 435/461; 435/320.1

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L50: Entry 54 of 69 File: USPT Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5854044 A
TITLE: Recombinant pseudomonas exotoxin with increased activity

Brief Summary Text (9):
The invention includes recombinant Pseudomonas exotoxin molecules in which domain Ia is deleted and no more than the first 27 amino acids from the amino terminal end of domain II have been deleted. A preferred PE molecule begins with a methionine at amino acid position 280 of domain II, comprises the deletion of about amino acids 365 to 380 of domain Ib and includes a substitution of serine at amino acid position position 287 in place of cysteine. Preferred molecules also include those that have an amino acid sequence at a carboxyl terminal end of the molecule selected from the group consisting of REDLK (Seq. ID No. 14), REDL (Seq. ID No. 15), and KDEL (Seq. ID No. 16). Exemplary PE molecules may consist essentially of about amino acids 280 to 613 or consist essentially of about amino acids 280 to 364 and 381 to 613.

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L32: Entry 27 of 52 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277413 B1
TITLE: Biodegradable compositions for the controlled release of encapsulated substances

CLAIMS:

1. A lipid/polymer-containing pharmaceutical composition comprising:

a biodegradable microsphere having a matrix, said matrix comprising at least one type of biodegradable polymer, and at least one type of lipid; and

a physiologically active substance which is releasable from the biodegradable microsphere.

2. The pharmaceutical composition of claim 1, wherein the microsphere is substantially free of volatile organic solvent.

3. The pharmaceutical composition of claim 1, wherein the microsphere is substantially free of poly vinyl alcohol.

5. The pharmaceutical composition of claim 4, wherein the solid dosage is selected from the group consisting of tablets, capsules, wafers, transdermal patches, sutures, implants, and suppositories.

17. The pharmaceutical composition of claim 1, wherein the physiologically active substance is selected from the group consisting of antianginas, antiarrhythmics, antiasthmatic agents, antibiotics, antidiabetics, antifungals, antihistamines, antihypertensives, antiparasitics, antineoplastics, antivirals, cardiac glycosides, herbicides, hormones, immunomodulators, monoclonal antibodies, neurotransmitters, nucleic acids, proteins, radio contrast agents, radionuclides, sedatives, analgesics, steroids, tranquilizers, vaccines, vasopressors, anesthetics, peptides, and combinations thereof.

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L6: Entry 12 of 23 File: USPT Dec 15, 1998

DOCUMENT-IDENTIFIER: US 5849993 A
TITLE: Transgenic mice as a model for metabolic bone diseases

CLAIMS:

1. A transgenic mouse displaying phenotypic characteristics indicative of metabolic bone disease, wherein said mouse lacks functional osteoblasts, wherein the genome of of said mouse comprises a recombinant DNA construct consisting of the gene encoding diphtheria toxin A chain operably linked to a rat osteocalcin promoter, and wherein expression of said diphtheria toxin A chain in the osteoblasts of said mouse results results in the genetic ablation of said osteoblasts in said mouse.

2. The transgenic mouse according to claim 1 characterized by a decrease in bone mass of the trabecula and bone cortex as well as by a reduction of the number of chondrocytes of the bone tissues of said mouse as a result of the expression of diphtheria toxin A chain in the osteoblasts of said mouse causing the genetic ablation of said osteoblasts in said mouse.

L32: Entry 25 of 52 File: USPT Nov 6, 2001

DOCUMENT-IDENTIFIER: US 6312731 B1
TITLE: Rapid release encapsulated bioactive agents for inducing or potentiating an immune response and methods of using thereof

CLAIMS:

1. A method of inducing or potentiating a CTL response in a subject, comprising administering to the subject an effective amount of a composition comprising an antigen or a nucleic acid encoding the antigen, encapsulated in a polymeric composition, wherein the polymeric composition comprises a blend of

(a) a polymer present in an amount sufficient to provide structural integrity to the polymeric composition, and

(b) a component selected from the group consisting of a rapidly biodegradable component, a rapidly dissolving component, a rapidly swelling component, and a component that causes osmotic rupture of the encapsulated polymeric composition thereby inducing or potentiating the CTL response in the subject.

2. The method of claim 1 wherein the polymeric composition provides kinetics of antigen or nucleic acid release such that a CTL response is measurable within fourteen days of a single administration at a level of at least 30% cytotoxicity.

16. The method of claim 1 wherein the polymeric composition is a microparticle, microcapsule, microsphere, nanoparticle, nanocapsule or nanosphere.

20. The method of claim 1 wherein the composition is administered rectally, vaginally, nasally, orally, opthamalically, transdermally, intradermally, topically, parenterally or by inhalation.

22. The method of claim 1 wherein the dosage of the antigen or nucleic acid encoding the antigen is from 1 ng to 100 mg.

24. A composition for inducing or potentiating a CTL response in a subject, comprising an antigen or a nucleic acid encoding the antigen, encapsulated in a polymeric composition, wherein the polymeric composition comprises a blend of

(a) a polymer present in an amount sufficient to provide structural integrity to the polymeric composition, and

(b) a component selected from the group consisting of a rapidly biodegradable component, a rapidly dissolving component, a rapidly swelling component, and a component that causes osmotic rupture of the encapsulated polymeric composition thereby inducing or potentiating the CTL response in the subject.

25. The composition of claim 24 wherein the polymeric composition provides kinetics of antigen or nucleic acid release such that a CTL response is measurable within fourteen days of a single administration at a level of at least 30% cytotoxicity.

40. The composition of claim 24 wherein the polymeric composition is a microparticle, microcapsule, microsphere, nanoparticle, nanocapsule or nanosphere.

44. The composition of claim 24 wherein the polymeric composition further comprises a pharmaceutically acceptable carrier.

45. A method of inducing or potentiating a T helper cell response in a subject, comprising administering to the subject an effective amount of a composition comprising an antigen or a nucleic acid encoding the antigen, encapsulated in a polymeric composition, wherein the polymeric composition comprises a blend of

(a) a polymer present in an amount sufficient to provide structural integrity to the polymeric composition, and

(b) a component selected from the group consisting of a rapidly biodegradable component, a rapidly dissolving component, a rapidly swelling component, and a component that causes osmotic rupture of the encapsulated polymeric composition.

46. The method of claim 45 wherein the polymeric composition provides kinetics of antigen or nucleic acid release such that a T helper cell response is measurable within fourteen days of a single administration at a level of at least two-fold over background as measured by T cell proliferation or cytokine induction, thereby inducing the T helper cell response in the subject.

47. A composition for inducing or potentiating a T helper cell response in a subject, comprising an antigen or a nucleic acid encoding the antigen, encapsulated in a polymeric composition, wherein the polymeric composition comprises a blend of

(a) a polymer present in an amount sufficient to provide structural integrity to the polymeric composition, and

(b) a component selected from the group consisting of a rapidly biodegradable component, a rapidly dissolving component, a rapidly swelling component, or a component that causes osmotic rupture of the encapsulated polymeric composition.

48. The composition of claim 47 wherein the polymeric composition provides kinetics of antigen or nucleic acid release such that a T helper cell response is measurable within fourteen days of a single administration at a level of at least two-fold over background.

49. A method of inducing or potentiating a neutralizing antibody response in a subject, comprising administering to the subject an effective amount of a composition comprising an antigen or a nucleic acid encoding the antigen, encapsulated in a polymeric composition, wherein the polymeric composition comprises a blend of

(a) a polymer present in an amount sufficient to provide structural integrity to the polymeric composition, and

(b) a component selected from the group consisting of a rapidly biodegradable component, a rapidly dissolving component, a rapidly swelling component, or a component that causes osmotic rupture of the encapsulated polymeric compositionthereby inducing the neutralizing antibody response in the subject.

50. The method of claim 49 wherein the polymeric composition provides kinetics of antigen or nucleic acid release such that the neutralizing antibody response is detectable within thirty days of a second or subsequent administration.

52. A composition for inducing or potentiating a neutralizing antibody response in a subject, comprising an antigen or a nucleic acid encoding the antigen,

encapsulated in a polymeric composition, wherein the polymeric composition comprises comprises a blend of

(a) a polymer present in an amount sufficient to provide structural integrity to the the polymeric composition, and

(b) a component selected from the group consisting of a rapidly biodegradable component, a rapidly dissolving component, a rapidly swelling component, and a component that causes osmotic rupture of the encapsulated polymeric composition.

53. The composition of claim 52 wherein the polymeric composition provides kinetics of antigen or nucleic acid release such that the neutralizing antibody response is detectable within thirty days of a second or subsequent administration.

56. The composition of claim 52 wherein the polymeric further comprises a pharmaceutically acceptable carrier.

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L32: Entry 24 of 52 File: USPT Dec 18, 2001

DOCUMENT-IDENTIFIER: US 6331310 B1
TITLE: Solid dose delivery vehicle and methods of making same

CLAIMS:

1. A device for the topical, subcutaneous, intradermal, or transdermal delivery of a a therapeutic agent, wherein the device includes a solid dose delivery vehicle comprising a therapeutically effective amount of said therapeutic agent and a polyol.

5. The device, according to claim 1, wherein the delivery vehicle is selected from the group consisting of needles, microneedles, microfibres, particles, microparticles, spheres, microspheres and powders of uniform particle size.

9. The device, according to claim 1, wherein said therapeutic agent is DNA.

14. A device for the topical, subcutaneous, intradermal, or transdermal delivery of a therapeutic agent, wherein the device includes a solid dose delivery vehicle comprising a therapeutically effective amount of said therapeutic agent and a polyol wherein said delivery of said therapeutic agent is via ballistic delivery.

18. The device, according to claim 14, wherein the delivery vehicle is selected from the group consisting of needles, microneedles, microfibres, particles, microparticles, spheres, microspheres and powders of uniform particle size.

22. The device, according to claim 14, wherein said therapeutic agent is DNA.

28. A method for the topical, subcutaneous, intradermal or transdermal delivery of a therapeutic agent to a subject, wherein the agent is in the form of a solid dose delivery vehicle comprising a therapeutically effective amount of said therapeutic agent and a polyol.

32. The method, according to claim 28, wherein the delivery vehicle is selected from the group consisting of needles, microneedles, microfibres, particles, microparticles, spheres, microspheres and powders of uniform particle size.

36. The method, according to claim 28, wherein said therapeutic agent is DNA.

41. A method for the topical, subcutaneous, intradermal or transdermal delivery of a therapeutic agent to a subject, wherein the agent is in the form of a solid dose delivery vehicle comprising a therapeutically effective amount of said therapeutic agent and a polyol, and wherein said transdermal delivery of said therapeutic agent is via ballistic delivery.

45. The method, according to claim 41, wherein the delivery vehicle is selected from the group consisting of needles, microneedles, microfibres, particles, microparticles, spheres, microspheres and powders of uniform particle size.

49. The method, according to claim 41, wherein said therapeutic agent is DNA.

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L20: Entry 11 of 32 File: USPT Aug 20, 2002

US-PAT-NO: 6436407
DOCUMENT-IDENTIFIER: US 6436407 B1

TITLE: Mutant enterotoxin effective as a non-toxic adjuvant

DATE-ISSUED: August 20, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Clements; John D.	New Orleans	LA		
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US-CL-CURRENT: 424/208.1; 424/184.1, 424/200.1, 424/234.1, 424/235.1, 424/236.1, 424/278.1, 424/282.1, 530/350, 530/825

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L32: Entry 13 of 52 File: USPT Jul 1, 2003

US-PAT-NO: 6586409
DOCUMENT-IDENTIFIER: US 6586409 B1

TITLE: Adjuvant compositions and methods for enhancing immune responses to polynucleotide-based vaccines

DATE-ISSUED: July 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wheeler; Carl J.	Poway	CA		

US-CL-CURRENT: 514/44; 424/450, 435/320.1, 435/455, 530/323, 560/155, 560/224, 560/252

CLAIMS:

What is claimed is:

1. A composition comprising an immunogen-encoding polynucleotide and an adjuvant composition comprising a (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and one or more co-lipids.

2. The composition of claim 1 wherein said immunogen-encoding polynucleotide is is DNA, RNA, or nucleic acid oligomer.

3. The composition of claim 1 wherein said immunogen-encoding polynucleotide is is a linear or circular polynucleotide.

4. The composition of claim 1 wherein said immunogen-encoding polynucleotide is is all or part of a plasmid DNA.

5. The composition of claim 1 wherein the co-lipid is selected from the group consisting of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine, and 1,2-dimyristoyl-glycero-3-phosphoethanolamine.

6. The composition of claim 5 wherein the co-lipid is 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine.

7. The composition of claim 5 wherein the co-lipid is 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine.

8. The composition of claim 1 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid lipid are in molar ratio of from about 9:1 to about 1:9.

9. The composition of claim 1 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid lipid are in molar ratio of from about 4:1 to about 1:4.

10. The composition of claim 1 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid lipid are in molar ratio of from about 2:1 to about 1:2.

11. The composition of claim 1 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid lipid are in molar ratio of about 1:1.

12. The composition of claim 7 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine are in molar ratio of from about 2:1 to about 1:2.

13. The composition of claim 7 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine are in molar ratio of about 1:1.

14. A method for increasing the immune response to an immunogen in a vertebrate vertebrate comprising administering into a tissue of said vertebrate an immunogenic composition comprising one or more immunogen-encoding polynucleotides and an adjuvant composition comprising a (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt, wherein said one or more polynucleotides are DNA, wherein said immunogen is expressed in the vertebrate in an amount sufficient to generate an immune response to said immunogen, and wherein said adjuvant composition increases said immune response.

15. The method of claim 14 wherein the immunogenic composition further comprises one or more co-lipids.

16. The method of claim 15 wherein the immunogen-encoding polynucleotide is all all or part of a plasmid DNA.

17. The method of claim 15 wherein the co-lipid is selected from the group consisting of 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine, and 1,2-dimyristoyl-glycero-3-phosphoethanolamine.

18. The method of claim 15 wherein the co-lipid is 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine.

19. The method of claim 15 wherein the co-lipid is 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine.

20. The method of claim 15 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid are in molar ratio of from about 9:1 to about 1:9.

21. The method of claim 15 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid are in molar ratio of from about 4:1 to about 1:4.

22. The method of claim 15 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid are in molar ratio of from about 2:1 to about 1:2.

23. The method of claim 15 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and the co-lipid are in molar ratio of about 1:1.

24. The method of claim 19 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine are in molar ratio of from about 2:1 to about 1:2.

25. The method of claim 19 wherein the (.+-.)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(syn-9-tetradeceneyloxy)-1-pr opanaminium salt and 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine are in molar ratio of about 1:1.

26. The method of claim 14 wherein the vertebrate is a mammal.

27. The method of claim 26 wherein the mammal is a human.

28. The method of claim 15 wherein the immunogenic composition is a pharmaceutical composition.

29. The method of claim 15 wherein said tissue is selected from the group consisting of muscle, skin, brain tissue, lung tissue, liver tissue, spleen tissue, bone marrow tissue, thymus tissue, heart tissue, lymph tissue, blood tissue, bone tissue, connective tissue, mucosal tissue, pancreas tissue, kidney kidney tissue, gall bladder tissue, stomach tissue, intestinal tissue, testicular tissue, ovarian tissue, uterine tissue, vaginal tissue, rectal tissue, nervous system tissue, eye tissue, glandular tissue, and tongue.

30. The method of claim 15, wherein said tissue is mucosal tissue, and wherein said composition is administered to the surface of said mucosal tissue.

31. The method of claim 15, wherein said tissue is muscle.

32. The method of claim 31, wherein said tissue is skeletal muscle.

33. The method of claim 15, wherein said administration is intravenous.

34. The method of claim 15, wherein said administration is by a route selected from the group consisting of intramuscular, intratracheal, intranasal, transdermal, interdermal, subcutaneous, intraocular, vaginal, rectal, intraperitoneal, intraintestinal and inhalation.

35. The method of any one of claim 15, wherein said administration is mediated by a device selected from the group consisting of a particle accelerator, a pump, an intradermal applicator, a biolistic injector, a pneumatic injector, a sponge depot, a pill and a tablet.

36. The method of claim 15, wherein said administration is mediated by a needle-free injection device.

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L32: Entry 27 of 52 File: USPT Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277413 B1
TITLE: Biodegradable compositions for the controlled release of encapsulated substances

CLAIMS:

1. A lipid/polymer-containing pharmaceutical composition comprising:

a biodegradable microsphere having a matrix, said matrix comprising at least one type of biodegradable polymer, and at least one type of lipid; and

a physiologically active substance which is releasable from the biodegradable microsphere.

2. The pharmaceutical composition of claim 1, wherein the microsphere is substantially free of volatile organic solvent.

3. The pharmaceutical composition of claim 1, wherein the microsphere is substantially free of poly vinyl alcohol.

5. The pharmaceutical composition of claim 4, wherein the solid dosage is selected from the group consisting of tablets, capsules, wafers, transdermal patches, sutures, implants, and suppositories.

17. The pharmaceutical composition of claim 1, wherein the physiologically active substance is selected from the group consisting of antianginas, antiarrhythmics, antiasthmatic agents, antibiotics, antidiabetics, antifungals, antihistamines, antihypertensives, antiparasitics, antineoplastics, antivirals, cardiac glycosides, herbicides, hormones, immunomodulators, monoclonal antibodies, neurotransmitters, nucleic acids, proteins, radio contrast agents, radionuclides, sedatives, analgesics, steroids, tranquilizers, vaccines, vasopressors, anesthetics, peptides, and combinations thereof.

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WEST Search History

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DATE: Thursday, January 06, 2005

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=AND	
☐	L1	first.clm. and second.clm. and third.clm.	502432
		DB=USPT; PLUR=YES; OP=AND	
☐	L2	adpribosylating.clm. or adp-ribosylating.clm. or cholera or (heat near labile) or exotoxin or exo-toxin or holotoxin or holo-toxin or ctx or (endotoxina or endotoxin-a or endo-toxin-a) or pertussis or diphtheria	11299
☐	L3	L2 near10 promoter$	149
☐	L4	L3 same (portion or domain or moiety or moieties or region or fragments or subunit or sub-unit or peptide)	94
☐	L5	expression.clm. same l2	32
☐	L6	L5 not l4	23

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Hide?	Set Name	Query	Hit Count
		DB=USPT; PLUR=YES; OP=AND	
☐	L1	(5416017 or 6033673).pn.	2
☐	L2	L1 and (na or nucleic or nucleotide or polynucleotide or dna or nuclear or dna or cdna or plasmid or vector or poly-nucleotide or mrna or c-dna)	2
☐	L3	(transdermal$ or trans-dermal$ or transmucosal$ or trans-mucosal$)	22153
☐	L4	L3 and l2	1
☐	L5	(6395964 or 5770203 or 5874287).pn.	3
☐	L6	L5 and l3	0
☐	L7	(adp or ribosylating or adp-ribosylating or exotoxin or exo-toxin or ribosyltransferase or ribosyl-transferase or cholera or hlt or hlt-a or hlta or hlt-b or hltb or lt or cta or ctb or ct-a or ct-b or ct or rct or rhlt)	179957
☐	L8	l7 and l3	4998
☐	L9	au or gold or carrier or particle or $particle or $carrier or tungsten or platinum or ferrite or polystyrene or poly-styrene or latex or beads or bead or $bead or $sphere or particles or partic$	6230295
☐	L10	L9 same l3	9011
☐	L11	L10 and l7	2113
☐	L12	L10 same l7	18
☐	L13	('6033645' |'5869057' |'6448073' |'6414139' |'5824538' |'6830924' |'6777546' |'6797276' |'6774226' |'6297364' |'6139819' |'6576756' |'6231834' |'5910306' |'6423705' |'6521211' |'5948407' |'5972376')!.PN.	18
☐	L14	(na or nucleic or nucleotide or polynucleotide or dna or nuclear or dna or cdna or plasmid or vector or poly-nucleotide or mrna or c-dna)	327578
☐	L15	L14 and l13	16
☐	L16	L15 and l3	16
☐	L17	l3 .ti,ab,clm.	2753
☐	L18	L17 and l9	2582
☐	L19	L18 and cholera	37
☐	L20	l19 and l14	32
☐	L21	l17 and l7.clm.	6
☐	L22	l3 same l14	820
☐	L23	L22 and l7	442
☐	L24	L23 an dl9	0

☐	L25	L23 and l9	437
☐	L26	l22 same l7	4
☐	L27	transcutaneous or trans-cutaneous or transskin or trans-skin	3013
☐	L28	L27 0r l3	0
☐	L29	l3L28	0
☐	L30	L29 or l3	22153
☐	L31	L30.clm.	2397
☐	L32	L31 and l14.clm. and l9.clm.	52
☐	L33	l30.clm. and (cholera or exotoxin or exo-toxin or ribosylat$ or $ribosyltransferase or adp).clm.	6
☐	L34	l14.clm. same l9.clm.	5213
☐	L35	(botulinum or c2 or c3 or pertussis or pt or lt1 or lt2 or endotoxin or exotoxin or epa or pseudomonas or clostridial or adpribosylating or cholera or diphtheria or ribosylating or ribosylation or adpribosyltransferase)	198180
☐	L36	L35.clm.	17723
☐	L37	l27 or l3	24625
☐	L38	L37.clm.	2826
☐	L39	L38 and l35.clm.	33
☐	L40	(dna or nucleic or nucleotide or polynucleotide or rna or nuclear or gene or genetic or vector or cdna or mrna or c-dna or coding).clm.	82256
☐	L41	L40 and l38	119
☐	L42	L41 and l36	7
☐	L43	cholera.clm. same nucleic.clm.	15
☐	L44	mammal$.clm. and bacteri$.clm. and promoter.clm.	272
☐	L45	L44 and $toxin.clm.	9
☐	L46	pseudomonas$.clm. same (moiety or domain or moieties or regions or portions or portion or sections or fragments or fragment or frag or domains).clm.	146
☐	L47	L46 same (dna or nucleic or nuclear or gene or rna or polynucleotide or nucleotide or nucleo-tide or cdna or mrna or c-dna).clm.	52
☐	L48	l47 and (kdel or rdel)	2
☐	L49	(kdel or rdel)	312
☐	L50	L49 same (deleted or delete or deletion or remove or removed or lack or lacks or substituted or substitution or substituting)	69
☐	L51	gene.clm. near2 therapy.clm.	159
☐	L52	L51 and adp.clm.	1
☐	L53	L51 and $toxin.clm.	10

END OF SEARCH HISTORY

Search in Swiss-Prot and TrEMBL for: adp ribosyltransferase

Swiss-Prot Release 45.5 of 04-Jan-2005
TrEMBL Release 28.5 of 04-Jan-2005

- Number of sequences found in Swiss-Prot$_{(49)}$ and TrEMBL$_{(57)}$: **106**
- Note that the selected sequences can be saved to a file to be later retrieved; to do so, go to the bottom of this page.
- For more directed searches, you can use the Sequence Retrieval System SRS.

Search in Swiss-Prot: There are matches to 49 out of 167089 entries

AEXT_AERSA (**Q93Q17**)
ADP-ribosyltransferase toxin aexT (EC 2.4.2.-) (Exoenzyme T) (aexT protein). {GENE: Name=aexT} - Aeromonas salmonicida

ALT_BPT2 (**Q38424**)
NAD--protein ADP-ribosyltransferase (EC 2.4.2.-) (Alt protein). {GENE: Name=ALT} - Bacteriophage T2

ALT_BPT4 (**P12726**)
NAD--protein ADP-ribosyltransferase precursor (EC 2.4.2.-) (Alt protein). {GENE: Name=ALT} - Bacteriophage T4

ALT_BPT6 (**Q38433**)
NAD--protein ADP-ribosyltransferase (EC 2.4.2.-) (Alt protein). {GENE: Name=ALT} - Bacteriophage T6

ARC3_CBCP (**Q00901**)
Mono-ADP-ribosyltransferase C3 precursor (EC 2.4.2.-) (Exoenzyme C3). - Clostridium botulinum C bacteriophage

ARC3_CBDP (**P15879**)
Mono-ADP-ribosyltransferase C3 precursor (EC 2.4.2.-) (Exoenzyme C3). {GENE: Name=C3} - Clostridium botulinum D bacteriophage

ARC3_CLOLM (**Q46134**)
Mono-ADP-ribosyltransferase C3 precursor (EC 2.4.2.-) (Exoenzyme C3). {GENE: Name=c3} - Clostridium limosum

CHTA_VIBCH (**P01555**)
Cholera enterotoxin, A chain precursor (NAD(+)--diphthamide ADP-ribosyltransferase) (EC 2.4.2.36) (Cholera enterotoxin A subunit) [Contains: Cholera enterotoxin chain-A1 (Cholera

enterotoxin alpha chain); Cholera enterotoxin chain-A2 (Cholera enterotoxin gamma chain)]. {GENE: Name=ctxA; Synonyms=toxA; OrderedLocusNames=VC1457} - Vibrio cholerae

DRAT_RHORU (**P14299**)
NAD(+)--dinitrogen-reductase ADP-D-ribosyltransferase (EC 2.4.2.37) (ADP-ribosyltransferase). {GENE: Name=draT} - Rhodospirillum rubrum

DTX_CORBE (**P00588**)
Diphtheria toxin precursor (DT) (NAD(+)--diphthamide ADP-ribosyltransferase) (EC 2.4.2.36). - Corynephage beta

DTX_COROM (**P00587**)
Diphtheria toxin precursor (DT) (NAD(+)--diphthamide ADP-ribosyltransferase) (EC 2.4.2.36). - Corynephage omega

MODA_BPT4 (**P39423**)
NAD--protein ADP-ribosyltransferase modA (EC 2.4.2.-) (RNA polymerase ADP-ribosylase modA). {GENE: Name=MODA} - Bacteriophage T4

MODB_BPT4 (**P39421**)
NAD--protein ADP-ribosyltransferase modB (EC 2.4.2.-). {GENE: Name=MODB; Synonyms=MOD} - Bacteriophage T4

NAR1_HUMAN (**P52961**)
GPI-linked NAD(P)(+)--arginine ADP-ribosyltransferase 1 precursor (EC 2.4.2.31) (Mono(ADP-ribosyl)transferase). {GENE: Name=ART1} - Homo sapiens (Human)

NAR1_MOUSE (**Q60935**)
GPI-linked NAD(P)(+)--arginine ADP-ribosyltransferase 1 precursor (EC 2.4.2.31) (Mono(ADP-ribosyl)transferase) (YAC-1). {GENE: Name=Art1; Synonyms=Art2} - Mus musculus (Mouse)

NAR1_RABIT (**Q03515**)
GPI-linked NAD(P)(+)--arginine ADP-ribosyltransferase 1 precursor (EC 2.4.2.31) (Mono(ADP-ribosyl)transferase). {GENE: Name=ART1} - Oryctolagus cuniculus (Rabbit)

NAR3_HUMAN (**Q13508**)
Ecto-ADP-ribosyltransferase 3 precursor (EC 2.4.2.31) (NAD(P)(+)--arginine ADP-ribosyltransferase 3) (Mono(ADP-ribosyl)transferase 3). {GENE: Name=ART3; Synonyms=TMART} - Homo sapiens (Human)

NAR3_MOUSE (**Q8R2G4**)
Ecto-ADP-ribosyltransferase 3 precursor (EC 2.4.2.31) (NAD(P)(+)--arginine ADP-ribosyltransferase 3) (Mono(ADP-ribosyl)transferase 3). {GENE: Name=Art3} - Mus musculus (Mouse)

NAR4_HUMAN (**Q93070**)
Ecto-ADP-ribosyltransferase 4 precursor (EC 2.4.2.31) (NAD(P)(+)--arginine ADP-ribosyltransferase 4) (Mono(ADP-ribosyl)transferase 4) (Dombrock blood group carrier molecule). {GENE: Name=DO; Synonyms=ART4, DOK1} - Homo sapiens (Human)

NAR4_PANTR (**Q95NE0**)
Ecto-ADP-ribosyltransferase 4 precursor (EC 2.4.2.31) (NAD(P)(+)--arginine ADP-ribosyltransferase 4) (Mono(ADP-ribosyl)transferase 4) (Dombrock molecule 1). {GENE: Name=DO; Synonyms=ART4} - Pan troglodytes (Chimpanzee)

NAR5_HUMAN (**Q96L15**)
Ecto-ADP-ribosyltransferase 5 precursor (EC 2.4.2.31) (NAD(P)(+)--arginine ADP-ribosyltransferase 5) (Mono(ADP-ribosyl)transferase 5). {GENE: Name=ART5} - Homo sapiens (Human)

NAR5_MOUSE (**P70352**)
Ecto-ADP-ribosyltransferase 5 precursor (EC 2.4.2.31) (NAD(P)(+)--arginine ADP-ribosyltransferase 5) (Mono(ADP-ribosyl)transferase 5) (YAC-2). {GENE: Name=Art5} - Mus musculus (Mouse)

NARA_MOUSE (**P17981**)

T-cell ecto-ADP-ribosyltransferase 1 precursor (EC 2.4.2.31) (T-cell NAD(P)(+)--arginine ADP-ribosyltransferase 1) (T-cell mono(ADP-ribosyl)transferase 1) (T-cell differentiation marker Rt6 homolog 1). {GENE: Name=Art2a; Synonyms=Rt6-1, Rt6.1} - Mus musculus (Mouse)

NARA_RAT (**P17982**)
T-cell ecto-ADP-ribosyltransferase 1 precursor (EC 2.4.2.31) (T-cell NAD(P)(+)--arginine ADP-ribosyltransferase 1) (T-cell mono(ADP-ribosyl)transferase 1) (Alloantigen Rt6.1) (T-cell surface protein Rt6.1). {GENE: Name=Art2a; Synonyms=Rt6-a} - Rattus norvegicus (Rat)

NARB_MOUSE (**O35975**)
T-cell ecto-ADP-ribosyltransferase 2 precursor (EC 2.4.2.31) (T-cell NAD(P)(+)--arginine ADP-ribosyltransferase 2) (T-cell mono(ADP-ribosyl)transferase 2) (T-cell differentiation marker Rt6 homolog 2). {GENE: Name=Art2b; Synonyms=Rt6-2, Rt6.2} - Mus musculus (Mouse)

NARB_RAT (**P20974**)
T-cell ecto-ADP-ribosyltransferase 2 precursor (EC 2.4.2.31) (T-cell NAD(P)(+)--arginine ADP-ribosyltransferase 2) (T-cell mono(ADP-ribosyl)transferase 2) (Alloantigen Rt6.2) (T-cell surface protein Rt6.2). {GENE: Name=Art2b; Synonyms=Rt6-b} - Rattus norvegicus (Rat)

NARE_CHICK (**Q92080**)
Erythroblast NAD(P)(+)--arginine ADP-ribosyltransferase precursor (EC 2.4.2.31) (Mono(ADP-ribosyl)transferase). {GENE: Name=MADPRT} - Gallus gallus (Chicken)

NRT1_CHICK (**P55806**)
NAD(P)(+)--arginine ADP-ribosyltransferase 1 precursor (EC 2.4.2.31) (Mono(ADP-ribosyl) transferase 1) (AT1). - Gallus gallus (Chicken)

NRT2_CHICK (**P55807**)
NAD(P)(+)--arginine ADP-ribosyltransferase 2 precursor (EC 2.4.2.31) (Mono(ADP-ribosyl) transferase 2) (AT2). - Gallus gallus (Chicken)

PPO2_HUMAN (**Q9UGN5**)
Poly [ADP-ribose] polymerase-2 (EC 2.4.2.30) (PARP-2) (NAD(+) ADP-ribosyltransferase-2) (Poly[ADP-ribose] synthetase-2) (pADPRT-2) (hPARP-2). {GENE: Name=PARP2; Synonyms=ADPRT2, ADPRTL2} - Homo sapiens (Human)

PPO2_MOUSE (**O88554**)
Poly [ADP-ribose] polymerase-2 (EC 2.4.2.30) (PARP-2) (NAD(+) ADP-ribosyltransferase-2) (Poly[ADP-ribose] synthetase-2) (pADPRT-2) (mPARP-2). {GENE: Name=Parp2; Synonyms=Adprt2, Adprtl2, Aspartl2} - Mus musculus (Mouse)

PPO3_HUMAN (**Q9Y6F1**)
Poly [ADP-ribose] polymerase-3 (EC 2.4.2.30) (PARP-3) (NAD(+) ADP-ribosyltransferase-3) (Poly[ADP-ribose] synthetase-3) (pADPRT-3) (hPARP-3) (IRT1). {GENE: Name=PARP3; Synonyms=ADPRT3, ADPRTL3} - Homo sapiens (Human)

PPOL_ARATH (**Q11207**)
Poly [ADP-ribose] polymerase (EC 2.4.2.30) (PARP) (ADPRT) (NAD(+) ADP-ribosyltransferase) (Poly[ADP-ribose] synthetase). {GENE: Name=APP; OrderedLocusNames=At4g02390; ORFNames=T14P8.19} - Arabidopsis thaliana (Mouse-ear cress)

PPOL_BOVIN (**P18493**)
Poly [ADP-ribose] polymerase-1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+) ADP-ribosyltransferase-1) (Poly[ADP-ribose] synthetase-1). {GENE: Name=PARP1; Synonyms=ADPRT} - Bos taurus (Bovine)

PPOL_CHICK (**P26446**)
Poly [ADP-ribose] polymerase-1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+) ADP-ribosyltransferase-1) (Poly[ADP-ribose] synthetase-1). {GENE: Name=PARP1; Synonyms=ADPRT} - Gallus gallus (Chicken)

PPOL_CRIGR (**Q9R152**)
Poly [ADP-ribose] polymerase-1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+) ADP-

ribosyltransferase-1) (Poly[ADP-ribose] synthetase-1). {GENE: Name=PARP1; Synonyms=ADPRT} - Cricetulus griseus (Chinese hamster)

PPOL_DROME (**P35875**)
Poly [ADP-ribose] polymerase (EC 2.4.2.30) (PARP) (ADPRT) (NAD(+) ADP-ribosyltransferase) (Poly[ADP-ribose] synthetase). {GENE: Name=Parp; ORFNames=CG40411} - Drosophila melanogaster (Fruit fly)

PPOL_HUMAN (**P09874**)
Poly [ADP-ribose] polymerase-1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+) ADP-ribosyltransferase-1) (Poly[ADP-ribose] synthetase-1). {GENE: Name=PARP1; Synonyms=ADPRT, PPOL} - Homo sapiens (Human)

PPOL_MOUSE (**P11103**)
Poly [ADP-ribose] polymerase-1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+) ADP-ribosyltransferase-1) (Poly[ADP-ribose] synthetase-1) (msPARP). {GENE: Name=Parp1; Synonyms=Adprp, Adprt, Adprt1} - Mus musculus (Mouse)

PPOL_ONCMA (**Q08824**)
Poly [ADP-ribose] polymerase (EC 2.4.2.30) (PARP) (ADPRT) (NAD(+) ADP-ribosyltransferase) (Poly[ADP-ribose] synthetase) (Fragment). {GENE: Name=PARP1; Synonyms=ADPRT} - Oncorhynchus masou (Cherry salmon) (Masu salmon)

PPOL_RAT (**P27008**)
Poly [ADP-ribose] polymerase-1 (EC 2.4.2.30) (PARP-1) (ADPRT) (NAD(+) ADP-ribosyltransferase-1) (Poly[ADP-ribose] synthetase-1). {GENE: Name=Parp1; Synonyms=Adprt} - Rattus norvegicus (Rat)

PPOL_SARPE (**Q11208**)
Poly [ADP-ribose] polymerase (EC 2.4.2.30) (PARP) (ADPRT) (NAD(+) ADP-ribosyltransferase) (Poly[ADP-ribose] synthetase). - Sarcophaga peregrina (Flesh fly) (Boettcherisca peregrina)

PPOL_XENLA (**P31669**)
Poly [ADP-ribose] polymerase (EC 2.4.2.30) (PARP) (ADPRT) (NAD(+) ADP-ribosyltransferase) (Poly[ADP-ribose] synthetase) (Fragment). - Xenopus laevis (African clawed frog)

PRSN_PIEBR (**Q9GV36**)
Pierisin (EC 2.4.2.-) (NAD--DNA ADP-ribosyltransferase) (Pierisin-2) (Pierisin-B). - Pieris brassicae (White butterfly)

PRSN_PIERA (**Q9U8Q4**)
Pierisin (EC 2.4.2.-) (NAD--DNA ADP-ribosyltransferase) (Pierisin-1). - Pieris rapae (Cabbage white butterfly)

PYRE_ACIAD (**Q6F6Z6**)
Orotate phosphoribosyltransferase (EC 2.4.2.10) (OPRT) (OPRTase). {GENE: Name=pyrE; OrderedLocusNames=ACIAD3525} - Acinetobacter sp. (strain ADP1)

TOX1_BORPE (**P04977**)
Pertussis toxin subunit 1 precursor (PTX S1) (Islet-activating protein S1) (IAP S1) (NAD-dependent ADP-ribosyltransferase) (EC 2.4.2.-). {GENE: Name=ptxA; OrderedLocusNames=BP3783} - Bordetella pertussis

TOXA_PSEAE (**P11439**)
Exotoxin A precursor (NAD-dependent ADP-ribosyltransferase) (EC 2.4.2.-). {GENE: Name=eta; OrderedLocusNames=PA1148} - Pseudomonas aeruginosa

TRPD_ACIAD (**P00500**)
Anthranilate phosphoribosyltransferase (EC 2.4.2.18). {GENE: Name=trpD; OrderedLocusNames=ACIAD2462} - Acinetobacter sp. (strain ADP1)

Search in TrEMBL: There are matches to 57 out of 1560235 entries

O32738
ADP-ribosyltransferase {GENE:Name=cdtA} - Clostridium difficile
O32739
ADP-ribosyltransferase {GENE:Name=cdtB} - Clostridium difficile
O46407
NAD(P)(+)--arginine ADP-ribosyltransferase (EC 2.4.2.31) (Fragment) {GENE:Name=ART2} - Oryctolagus cuniculus (Rabbit)
O46408
NAD(P)(+)--arginine ADP-ribosyltransferase (EC 2.4.2.31) (Fragment) {GENE:Name=ART4} - Oryctolagus cuniculus (Rabbit)
O54738
NAD(P)(+)--arginine ADP-ribosyltransferase (EC 2.4.2.31) {GENE:Name=ART3} - Mus musculus (Mouse)
O54739
NAD(P)(+)--arginine ADP-ribosyltransferase (EC 2.4.2.31) {GENE:Name=Art5; Synonyms=ART5} - Mus musculus (Mouse)
Q44083
Dinitrogenase reductase ADP-ribosyltransferase {GENE:Name=draT} - Azospirillum lipoferum
Q45845
ADP-ribosyltransferase C3 - Clostridium botulinum D
Q52690
Dinitrogenase reductase ADP-ribosyltransferase {GENE:Name=draT} - Rhodobacter capsulatus (Rhodopseudomonas capsulata)
Q5RHR0
Novel protein similar to vertebrate ADP-ribosyltransferase (NAD+\; poly (ADP-ribose) polymerase) (ADPRT) {GENE:Name=OTTDARP00000006993; ORFNames=DKEY-206F10.3-001} - Brachydanio rerio (Zebrafish) (Danio rerio)
Q5UQA6
ADP-ribosyltransferase (DraT) {GENE:ORFNames=MIMI_L543} - Mimivirus
Q5VX84
ADP-ribosyltransferase (NAD+\; poly (ADP-ribose) polymerase) {GENE:Name=ADPRT; ORFNames=RP11-125A15.2-005} - Homo sapiens (Human)
Q5VX85
ADP-ribosyltransferase (NAD+\; poly (ADP-ribose) polymerase) {GENE:Name=ADPRT; ORFNames=RP11-125A15.2-003} - Homo sapiens (Human)
Q5XDK2
C3 family ADP-ribosyltransferase (EC 2.4.2.-) {GENE:ORFNames=M6_Spy0376} - Streptococcus pyogenes (serotype M6)
Q607D2
Dinitrogenase reductase ADP-ribosyltransferase-like protein {GENE:OrderedLocusNames=MCA1828} - Methylococcus capsulatus
Q675Z0
NAD(+) ADP-ribosyltransferase-3-like protein {GENE:ORFNames=003-16} - Oikopleura dioica
Q6F6M0
Hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) {GENE:Name=hpt; OrderedLocusNames=ACIAD3669} - Acinetobacter sp. (strain ADP1)
Q6F6W1
Nicotinate phosphoribosyltransferase (EC 2.4.2.11) {GENE:Name=pncB; OrderedLocusNames=ACIAD3562} - Acinetobacter sp. (strain ADP1)

Q6F7W2
Xanthine phosphoribosyltransferase (EC 2.4.2.22) {GENE:Name=xpt; OrderedLocusNames=ACIAD3164} - Acinetobacter sp. (strain ADP1)
Q6FCL7
Amidophosphoribosyltransferase (EC 2.4.2.14) {GENE:Name=purF; OrderedLocusNames=ACIAD1323} - Acinetobacter sp. (strain ADP1)
Q6FCS8
ATP phosphoribosyltransferase (EC 2.4.2.17) {GENE:Name=hisZ; OrderedLocusNames=ACIAD1257} - Acinetobacter sp. (strain ADP1)
Q6FE58
Uracil phosphoribosyltransferase (EC 2.4.2.9) {GENE:Name=upp; OrderedLocusNames=ACIAD0744} - Acinetobacter sp. (strain ADP1)
Q6FEC9
ATP-phosphoribosyltransferase (EC 2.4.2.17) {GENE:Name=hisG; OrderedLocusNames=ACIAD0661} - Acinetobacter sp. (strain ADP1)
Q6FEJ3
S-adenosylmethionine:tRNA ribosyltransferase-isomerase (Queuosine biosynthesis protein) (EC 5.-.-.-) {GENE:Name=queA; OrderedLocusNames=ACIAD0591} - Acinetobacter sp. (strain ADP1)
Q6FEJ4
Queuine tRNA-ribosyltransferase (TRNA-guanine transglycosylase) (Guanine insertion enzyme) (EC 2.4.2.29) {GENE:Name=tgt; OrderedLocusNames=ACIAD0590} - Acinetobacter sp. (strain ADP1)
Q6FFW3
Nicotinate-nucleotide pyrophosphorylase (Quinolinate phosphoribosyltransferase) (EC 2.4.2.19) {GENE:Name=nadC; OrderedLocusNames=ACIAD0062} - Acinetobacter sp. (strain ADP1)
Q6LAL6
NAD(P)(+)--arginine ADP-ribosyltransferase (EC 2.4.2.31) (Fragment) {GENE:Name=ATR4} - Rattus norvegicus (Rat)
Q6N6U0
Sir2 family, possible ADP ribosyltransferase {GENE:OrderedLocusNames=RPA2524} - Rhodopseudomonas palustris
Q6N757
NAD+ ADP-ribosyltransferase (EC 2.4.2.30) {GENE:Name=draT2; OrderedLocusNames=RPA2405} - Rhodopseudomonas palustris
Q6N9V5
Putative NAD+ ADP-ribosyltransferase {GENE:Name=draT1; OrderedLocusNames=RPA1431} - Rhodopseudomonas palustris
Q6NTD2
ADP-ribosyltransferase 1 {GENE:Name=ART1} - Homo sapiens (Human)
Q70MS6
Dinitrogenase reductase ADP-ribosyltransferase (Fragment) {GENE:Name=draT} - Gigaspora margarita
Q749E1
NAD(+)--dinitrogen-reductase ADP-D-ribosyltransferase (EC 2.4.2.37) {GENE:Name=dRAT; OrderedLocusNames=GSU2802} - Geobacter sulfurreducens
Q7WUH3
Binary ADP-ribosyltransferase CDT toxin {GENE:Name=cdt} - Clostridium difficile
Q7Z114
NAD(+) ADP-ribosyltransferase-4 {GENE:Name=adprt-4} - Dictyostelium discoideum (Slime mold)

Q7Z115
NAD(+) ADP-ribosyltransferase-1A (EC 2.4.2.30) {GENE:Name=adprt-1A} - Dictyostelium discoideum (Slime mold)
Q7ZVB0
ADP-ribosyltransferase (NAD+; poly (ADP-ribose polymerase)-like 3) {GENE:ORFNames=zgc:66157} - Brachydanio rerio (Zebrafish) (Danio rerio)
Q80VB5
NAD(P)(+)--arginine ADP-ribosyltransferase (EC 2.4.2.31) (Fragment) {GENE:Name=Art4; Synonyms=ART4} - Mus musculus (Mouse)
Q8BPD2
Mus musculus 18 days pregnant adult female placenta and extra embryonic tissue cDNA, RIKEN full-length enriched library, clone:3830425K23 product:ADP-ribosyltransferase (NAD+; poly (ADP-ribose) polymerase) 2, full insert sequence - Mus musculus (Mouse)
Q8CFB6
NAD+ ADP-ribosyltransferase 3 PARP-3 (Fragment) - Mus musculus (Mouse)
Q8CFB8
NAD+ ADP-ribosyltransferase 3 PARP-3 {GENE:Name=Adprtl3} - Mus musculus (Mouse)
Q8I7C4
NAD(+) ADP-ribosyltransferase-3 {GENE:Name=adprt-3} - Dictyostelium discoideum (Slime mold)
Q8I7C5
NAD(+) ADP-ribosyltransferase-1B {GENE:Name=adprt-1B} - Dictyostelium discoideum (Slime mold)
Q8KNY0
ADP-ribosyltransferase (Fragment) {GENE:Name=c3cer} - Bacillus cereus
Q8X0P2
Related to NAD+ ADP-ribosyltransferase {GENE:Name=5E6.250} - Neurospora crassa
Q977Y7
Mono-ADP-ribosyltransferase C3; CF-7 family {GENE:OrderedLocusNames=CAC0338} - Clostridium acetobutylicum
Q9ADS9
ADP-ribosyltransferase (Fragment) - Staphylococcus aureus
Q9CRA0
Mus musculus 14 days embryo liver cDNA, RIKEN full-length enriched library, clone:4432404K01 product:similar to MONO (ADP-RIBOSYL)TRANSFERASE (ADP-ribosyltransferase 4) (Mus musculus adult male lung cDNA, RIKEN full-length enriched library, clone:1200013G08 product:similar to MONO (ADP-RIBOSYL)TRANSFERASE) {GENE:Name=Art4} - Mus musculus (Mouse)
Q9D7F1
Mus musculus adult male tongue cDNA, RIKEN full-length enriched library, clone:2310010O08 product:ADP-ribosyltransferase 1, full insert sequence - Mus musculus (Mouse)
Q9JP48
Dinitrogenase reductase ADP-ribosyltransferase {GENE:Name=draT} - Azospirillum brasilense
Q9R654
ADP-RIBOSYLTRANSFERASE=RHO-ADP-RIBOSYLATING exoenzyme (Fragments) - Bacillus cereus
Q9RM75
Clostridium difficile binary toxin A (Actin-specific adp-ribosyltransferase) (Fragment) {GENE:Name=cdtA} - Clostridium difficile
Q9RM76
Clostridium difficile binary toxin A (Actin-specific adp-ribosyltransferase) (Fragment)

{GENE:Name=cdtA} - Clostridium difficile
Q9RM77
ADP-ribosyltransferase (Fragment) {GENE:Name=cdtB} - Clostridium difficile
Q9RM78
ADP-ribosyltransferase (Fragment) {GENE:Name=cdtB} - Clostridium difficile
Q9RM79
ADP-ribosyltransferase (Fragment) {GENE:Name=cdtB} - Clostridium difficile
Q9RM80
ADP-ribosyltransferase (Fragment) {GENE:Name=cdtB} - Clostridium difficile

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New Search
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Please do NOT use any boolean operators (and, or, etc.)

If you would like to retrieve all the Swiss-Prot/TrEMBL entries contained in this list , you can enter a file name. These entries will then be saved to a file under this name in the directory `outgoing` of the ExPASy anonymous ftp server, from where you can download it. (Please note that this temporary file will only be kept for 1 week.)

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Search Results - Record(s) 1 through 18 of 18 returned.

L13: Entry 1 of 18 File: USPT Dec 14, 2004

US-PAT-NO: 6830924
DOCUMENT-IDENTIFIER: US 6830924 B1

TITLE: Isolated nucleic acid molecule encoding cancer associated antigens, the antigens per se, and uses thereof

DATE-ISSUED: December 14, 2004

US-CL-CURRENT: 435/320.1; 435/252.3, 435/254.11, 435/325, 536/23.5, 536/24.33

INT-CL: [07] C12 N 15/63, C12 N 5/16, C12 N 1/20, C12 N 1/21, C07 H 21/04

L13: Entry 2 of 18 File: USPT Sep 28, 2004

US-PAT-NO: 6797276
DOCUMENT-IDENTIFIER: US 6797276 B1

TITLE: Use of penetration enhancers and barrier disruption agents to enhance the transcutaneous immune response

DATE-ISSUED: September 28, 2004

US-CL-CURRENT: 424/278.1; 424/184.1, 424/204.1, 424/206.1, 424/234.1, 424/241.1, 424/265.1, 424/274.1, 424/283.1

INT-CL: [07] A61 K 45/00, A61 K 47/00

L13: Entry 3 of 18 File: USPT Aug 17, 2004

US-PAT-NO: 6777546
DOCUMENT-IDENTIFIER: US 6777546 B2

TITLE: Methods and substances for preventing and treating autoimmune disease

DATE-ISSUED: August 17, 2004

US-CL-CURRENT: 536/23.4; 435/419, 435/468, 435/69.3

INT-CL: [07] C07 H 21/04, C12 N 15/09, C12 N 5/04, C12 N 15/82

L13: Entry 4 of 18 File: USPT Aug 10, 2004

US-PAT-NO: 6774226
DOCUMENT-IDENTIFIER: US 6774226 B1

TITLE: Isolated nucleic acid molecules encoding cancer associated antigens, the antigens per se, and uses thereof

DATE-ISSUED: August 10, 2004

US-CL-CURRENT: 536/23.5; 435/320.1, 435/325

INT-CL: [07] C07 H 21/04, C12 N 15/00, C12 N 5/00

L13: Entry 5 of 18 File: USPT Jun 10, 2003

US-PAT-NO: 6576756
DOCUMENT-IDENTIFIER: US 6576756 B2

TITLE: Isolated nucleic acid molecule encoding cancer associated antigen, the antigen itself, and uses thereof

DATE-ISSUED: June 10, 2003

US-CL-CURRENT: 536/23.5; 530/350

INT-CL: [07] C07 H 21/04, C07 K 1/00, A01 N 37/18

L13: Entry 6 of 18 File: USPT Feb 18, 2003

US-PAT-NO: 6521211
DOCUMENT-IDENTIFIER: US 6521211 B1

TITLE: Methods of imaging and treatment with targeted compositions

DATE-ISSUED: February 18, 2003

US-CL-CURRENT: 424/9.52; 424/450, 424/9.5, 424/9.51, 514/18, 514/2, 600/431, 600/437

INT-CL: [07] A61 B 8/00, A61 R 9/127, A61 R 38/00, A61 R 38/04

L13: Entry 7 of 18 File: USPT Sep 10, 2002

US-PAT-NO: 6448073
DOCUMENT-IDENTIFIER: US 6448073 B1
**** See image for Certificate of Correction ****

TITLE: Isolated nucleic acid molecules encoding cancer associated antigens, the antigens per se, and uses thereof

DATE-ISSUED: September 10, 2002

US-CL-CURRENT: 435/320.1; 435/252.3, 435/325, 435/366, 536/23.1, 536/23.5, 536/24.5

INT-CL: [07] C12 N 15/63, C12 N 5/00, C12 N 1/20, C07 H 21/04

L13: Entry 8 of 18 File: USPT Jul 23, 2002

US-PAT-NO: 6423705
DOCUMENT-IDENTIFIER: US 6423705 B1

TITLE: Combination therapy

DATE-ISSUED: July 23, 2002

US-CL-CURRENT: 514/221; 514/252.13, 514/331, 514/634

INT-CL: [07] A61 K 31/55, A61 K 31/495, A61 K 31/445, A61 K 31/155

L13: Entry 9 of 18 File: USPT Jul 2, 2002

US-PAT-NO: 6414139
DOCUMENT-IDENTIFIER: US 6414139 B1
**** See image for Certificate of Correction ****

TITLE: Silicon amphiphilic compounds and the use thereof

DATE-ISSUED: July 2, 2002

US-CL-CURRENT: 556/413; 556/404, 556/405, 556/425, 556/427, 556/428, 556/436, 556/437

INT-CL: [07] C07 F 7/10

L13: Entry 10 of 18 File: USPT Oct 2, 2001

US-PAT-NO: 6297364
DOCUMENT-IDENTIFIER: US 6297364 B1

TITLE: Isolated nucleic acid molecule encoding cancer associated antigen, the antigen itself, and uses thereof

DATE-ISSUED: October 2, 2001

US-CL-CURRENT: 536/23.1; 424/277.1, 435/320.1, 435/325

INT-CL: [07] C07 H 21/02, C12 N 15/00, C12 N 5/00, A61 K 39/00

L13: Entry 11 of 18 File: USPT May 15, 2001

US-PAT-NO: 6231834
DOCUMENT-IDENTIFIER: US 6231834 B1
**** See image for Certificate of Correction ****

TITLE: Methods for ultrasound imaging involving the use of a contrast agent and multiple images and processing of same

DATE-ISSUED: May 15, 2001

US-CL-CURRENT: 424/9.51; 424/9.52, 600/431

INT-CL: [07] A61 B 8/13

L13: Entry 12 of 18 File: USPT Oct 31, 2000

US-PAT-NO: 6139819
DOCUMENT-IDENTIFIER: US 6139819 A
**** See image for Certificate of Correction ****

TITLE: Targeted contrast agents for diagnostic and therapeutic use

DATE-ISSUED: October 31, 2000

US-CL-CURRENT: 424/9.52; 424/450, 424/9.51

INT-CL: [07] A61 B 8/00, A61 K 9/127

L13: Entry 13 of 18 File: USPT Mar 7, 2000

US-PAT-NO: 6033645
DOCUMENT-IDENTIFIER: US 6033645 A
**** See image for Certificate of Correction ****

TITLE: Methods for diagnostic imaging by regulating the administration rate of a contrast agent

DATE-ISSUED: March 7, 2000

US-CL-CURRENT: 424/9.5; 424/450, 424/9.51, 424/9.52

INT-CL: [07] A61 K 49/00

L13: Entry 14 of 18 File: USPT Oct 26, 1999

US-PAT-NO: 5972376
DOCUMENT-IDENTIFIER: US 5972376 A

TITLE: Transdermal system of tacrine/selegilin-plaster

DATE-ISSUED: October 26, 1999

US-CL-CURRENT: 424/449; 424/448

INT-CL: [06] A61 F 13/00

L13: Entry 15 of 18 File: USPT Sep 7, 1999

US-PAT-NO: 5948407
DOCUMENT-IDENTIFIER: US 5948407 A

TITLE: Oral induction of tolerance to parenterally administered non-autologous polypeptides

DATE-ISSUED: September 7, 1999

US-CL-CURRENT: 424/184.1; 424/520, 424/810, 424/93.1, 514/2, 514/21, 514/3, 514/8, 530/303, 530/350, 530/383, 530/384

INT-CL: [06] A01 N 63/00, A61 K 38/37, A61 K 35/02, A61 K 38/28

US-PAT-NO: 5910306
DOCUMENT-IDENTIFIER: US 5910306 A

TITLE: Transdermal delivery system for antigen

DATE-ISSUED: June 8, 1999

US-CL-CURRENT: 424/184.1; 424/204.1, 424/234.1, 424/265.1, 424/269.1, 424/274.1, 424/277.1, 424/279.1, 424/282.1, 424/283.1, 424/449, 424/450, 424/810, 424/812

INT-CL: [06] A61 K 39/00, A61 K 39/002, A61 K 39/02, A61 K 39/12

L13: Entry 17 of 18 File: USPT Feb 9, 1999

US-PAT-NO: 5869057
DOCUMENT-IDENTIFIER: US 5869057 A

TITLE: Recombinant vaccines to break self-tolerance

DATE-ISSUED: February 9, 1999

US-CL-CURRENT: 424/192.1; 435/69.3, 530/403, 536/23.5, 536/23.7

INT-CL: [06] A61 K 39/108, C07 K 14/245, C07 H 21/02

L13: Entry 18 of 18 File: USPT Oct 20, 1998

US-PAT-NO: 5824538
DOCUMENT-IDENTIFIER: US 5824538 A

TITLE: Shigella vector for delivering DNA to a mammalian cell

DATE-ISSUED: October 20, 1998

US-CL-CURRENT: 435/252.1; 424/93.2, 435/245, 435/252.3, 435/455, 435/822

INT-CL: [06] C12 N 1/00, C12 N 1/20, C12 N 15/00

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NiceProt View of Swiss-Prot: P11439

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**TOXA_PSEAE**
Primary accession number	**P11439**
Secondary accession number	Q9I4I7
Entered in Swiss-Prot in	Release 12, October 1989
Sequence was last modified in	Release 40, October 2001
Annotations were last modified in	Release 46, January 2005

Name and origin of the protein

Protein name	**Exotoxin A [Precursor]**
Synonyms	**NAD-dependent ADP-ribosyltransferase** **EC 2.4.2.-**
Gene name	**Name: eta** OrderedLocusNames: PA1148
From	Pseudomonas aeruginosa [TaxID: 287]
Taxonomy	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.

References

[1] NUCLEOTIDE SEQUENCE, AND PROTEIN SEQUENCE OF 26-53.
MEDLINE=84194063;PubMed=6201861 [NCBI, ExPASy, EBI, Israel, Japan]
Gray G.L., Smith D.H., Baldridge J.S., Harkins R.N., Vasil M.L., Chen E.Y., Heyneker H.L.;
"Cloning, nucleotide sequence, and expression in Escherichia coli of the exotoxin A structural gene of Pseudomonas aeruginosa.";
Proc. Natl. Acad. Sci. U.S.A. 81:2645-2649(1984).

[2] NUCLEOTIDE SEQUENCE [LARGE SCALE GENOMIC DNA].
STRAIN=ATCC 15692 / PAO1;
DOI=10.1038/35023079;MEDLINE=20437337;PubMed=10984043 [NCBI, ExPASy, EBI, Israel, Japan]
Stover C.K., Pham X.-Q.T., Erwin A.L., Mizoguchi S.D., Warrener P., Hickey M.J., Brinkman F.S.L., Hufnagle W.O., Kowalik D.J., Lagrou M., Garber R.L., Goltry L., Tolentino E., Westbrock-Wadman S., Yuan Y., Brody L.L., Coulter S.N., Folger K.R., Kas A., , Olson M.V.;

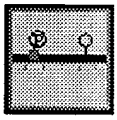
EMBL	K01397; AAB59097.1; -. [EMBL / GenBank / DDBJ] [CoDingSequence] AE004544; AAG04537.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	A30347; A30347. C83503; C83503.
PDB	1AER; X-ray; A=425-634, B=425-625.[ExPASy / RCSB / EBI] 1DMA; X-ray; A/B=425-638. [ExPASy / RCSB / EBI] 1IKP; X-ray; A=26-638. [ExPASy / RCSB / EBI] 1IKQ; X-ray; A=26-638. [ExPASy / RCSB / EBI] Detailed list of linked structures.
SWISS-3DIMAGE	P11439.
CMR	P11439; PA1148.
InterPro	IPR008985; ConA_like_lec_gl. Graphical view of domain structure.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
HOBACGEN	[Family / Alignment / Tree]
BLOCKS	P11439.
ProtoNet	P11439.
ProtoMap	P11439.
PRESAGE	P11439.
DIP	P11439.
ModBase	P11439.
SMR	P11439; 7B9AAD56A27C700A.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Pseudomonas Exotoxin A

Keywords

3D-structure; **Complete proteome**; **Direct protein sequencing**; **Glycosyltransferase**; **NAD**; **Signal**; **Toxin**; **Transferase**.

Features

 Feature table viewer

 Feature aligner

Key	From	To	Length	Description
SIGNAL	1	25	25	
CHAIN	26	638	613	Exotoxin A.
DOMAIN	26	277	252	IA (required for target cell recognition).
DOMAIN	278	389	112	II (required for translocation in target cell cytoplasm).
DOMAIN	390	429	40	IB.
DOMAIN	430	638	209	III (required for ADP-ribosyl activity).
NP_BIND	465	481	17	NAD.
ACT_SITE	578	578		
DISULFID	290	312		
CONFLICT	4	4		T -> I (in Ref. 1).
CONFLICT	22	22		F -> S (in Ref. 1).
CONFLICT	204	204		A -> T (in Ref. 1).
CONFLICT	389	389		S -> N (in Ref. 1).

CONFLICT	432	432		I -> V (in Ref. 1).
CONFLICT	540	540		G -> S (in Ref. 1).
STRAND	29	29	1	
HELIX	32	35	4	
STRAND	39	43	5	
TURN	45	46	2	
STRAND	49	54	6	
HELIX	57	60	4	
TURN	61	61	1	
STRAND	65	74	10	
TURN	76	79	4	
STRAND	81	85	5	
TURN	86	88	3	
STRAND	89	93	5	
STRAND	97	102	6	
STRAND	110	115	6	
STRAND	122	131	10	
TURN	132	133	2	
STRAND	137	145	9	
TURN	147	148	2	
STRAND	151	154	4	
STRAND	157	161	5	
HELIX	164	170	7	
TURN	171	172	2	
STRAND	173	180	8	
STRAND	189	201	13	
HELIX	213	216	4	
HELIX	218	223	6	
HELIX	225	227	3	
TURN	228	229	2	
HELIX	230	235	6	
HELIX	243	246	4	
TURN	247	247	1	
STRAND	249	255	7	
STRAND	262	262	1	
STRAND	270	273	4	
TURN	276	277	2	
HELIX	280	290	11	
TURN	291	291	1	
HELIX	294	298	5	
HELIX	307	311	5	
TURN	312	312	1	
HELIX	313	325	13	
TURN	326	327	2	
HELIX	330	332	3	
HELIX	333	342	10	
TURN	344	347	4	
HELIX	348	356	9	

HELIX	358	376	19
TURN	377	378	2
TURN	380	381	2
HELIX	384	387	4
TURN	388	389	2
STRAND	392	396	5
HELIX	408	410	3
TURN	411	412	2
STRAND	414	418	5
HELIX	422	424	3
TURN	436	437	2
TURN	440	441	2
HELIX	444	456	13
TURN	457	458	2
STRAND	459	467	9
HELIX	469	477	9
TURN	478	478	1
HELIX	489	491	3
STRAND	494	497	4
HELIX	500	504	5
TURN	505	506	2
STRAND	508	508	1
TURN	514	515	2
STRAND	520	520	1
STRAND	522	529	8
HELIX	530	535	6
STRAND	536	538	3
TURN	543	544	2
TURN	546	547	2
HELIX	548	556	9
TURN	557	557	1
STRAND	566	570	5
TURN	573	574	2
STRAND	577	581	5
HELIX	583	587	5
TURN	588	588	1
STRAND	590	593	4
TURN	600	601	2
TURN	603	604	2
HELIX	609	611	3
HELIX	614	617	4
TURN	618	619	2
STRAND	626	626	1

Sequence information

Length: **638 AA** [This is the length of the unprocessed precursor]

Molecular weight: **69284 Da** [This is the MW of the unprocessed precursor]

CRC64: **7B9AAD56A27C700A** [This is a checksum on the sequence]

10 20 30 40 50 60

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 MHLTPHWIPL VASLGLLAGG SFASAAEEAF DLWNECAKAC VLDLKDGVRS SRMSVDPAIA

        70         80         90        100        110        120
DTNGQGVLHY SMVLEGGNDA LKLAIDNALS ITSDGLTIRL EGGVEPNKPV RYSYTRQARG

       130        140        150        160        170        180
SWSLNWLVPI GHEKPSNIKV FIHELNAGNQ LSHMSPIYTI EMGDELLAKL ARDATFFVRA

       190        200        210        220        230        240
HESNEMQPTL AISHAGVSVV MAQAQPRREK RWSEWASGKV LCLLDPLDGV YNYLAQQRCN

       250        260        270        280        290        300
LDDTWEGKIY RVLAGNPAKH DLDIKPTVIS HRLHFPEGGS LAALTAHQAC HLPLETFTRH

       310        320        330        340        350        360
RQPRGWEQLE QCGYPVQRLV ALYLAARLSW NQVDQVIRNA LASPGSGGDL GEAIREQPEQ

       370        380        390        400        410        420
ARLALTLAAA ESERFVRQGT GNDEAGAASA DVVSLTCPVA AGECAGPADS GDALLERNYP

       430        440        450        460        470        480
TGAEFLGDGG DISFSTRGTQ NWTVERLLQA HRQLEERGYV FVGYHGTFLE AAQSIVFGGV

       490        500        510        520        530        540
RARSQDLDAI WRGFYIAGDP ALAYGYAQDQ EPDARGRIRN GALLRVYVPR SSLPGFYRTG

       550        560        570        580        590        600
LTLAAPEAAG EVERLIGHPL PLRLDAITGP EEEGGRLETI LGWPLAERTV VIPSAIPTDP

       610        620        630
RNVGGDLDPS SIPDKEQAIS ALPDYASQPG KPPREDLK
```

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Comments

- ***FUNCTION***: This toxin is a NAD-dependent ADP-ribosyltransferase. It catalyzes the transfer of the ADP ribosyl moiety of oxidized NAD onto elongation factor 2 (EF-2) thus arresting protein synthesis.
- ***PTM***: The 8 cysteines participate in intrachain disulfide bonds.

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Cross-references